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Blacksburg

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Part II Viruses

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This study reports data from a two-year monitoring effort (1975-77) for several health-related constituents of drinking water in a northern Virginia system (Fairfax County Water Authority) which serves about 640,000 residents. This study addresses a broad range of interests with respect to viruses in detail and depth, with respect to a single, urbanizing reservoir and water service area. Since this project involved not only environmental studies but also a major basic testing of comparative virus sampling, concentrating and analytical schemes, its results will be of interest not only to water supply engineers but also to virologists and other scientists concerned with virus measurement technology and assessments. Within the limitations of the measurement technology, it also provides information with respect to the impact (or lack of impact) of wastewater and urbanizing factors on virus levels in natural untreated waters.

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PART II: VIRUSES

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FOREWORD

The Office of Drinking Water has broad interests in all aspects of rendering a safe drinking water for the American public. These interests run from questions of wastewater and urban impacts on raw water quality to the effects of treatment and the quality of distributed water.

The part of the "Occoquan" project reported herein addresses this broad range of interests with respect to viruses in detail and depth, with respect to a single, urbanizing reservoir and water service area. Since this project involved not only environmental studies but also a major basic testing of comparative virus sampling, concentrating and analytical schemes, its results will be of interest not only to water supply engineers but also to virologists and other scientists concerned with virus measurement technology and assessments. Within the limitations of the measurement technology, it also provides information with respect to the impact (or lack of impact) of wastewater and urbanizing factors on virus levels in natural untreated waters.

The Office of Drinking Water also wishes to recognize the extensive cooperation and participation of EPA's Office of Research and Development virus research group in Cincinnati, Ohio, without whom the entire project could not have been completed.

Joseph A. Cotruvo, Ph.D.
Director, Criteria and Standards Division
Office of Drinking Water

PREFACE

The project discussed in this report was begun soon after the passage of the Safe Drinking Water Act in 1974 (P. L. 93-523) and the completion of the EPA's National Organics Reconnaissance Survey (NORS) involving 80 cities in the United States. Late in 1974, the public began to be made aware that drinking water supplies may be contaminated with hazardous organic chemicals contributed by industries and created during the disinfection process. Concern also was expressed about the potential impact of urbanization and treated wastewater discharges on virus levels in raw sources for drinking water supplies. Extensive coverage by the news media fostered public interest in the subject, and because the data were so limited at the time, EPA immediately responded by conducting in-house and extramural research to determine the extent of the problem and how best to solve it. Since then, much has been accomplished.

This project, referred to as the Occoquan Report, carried out in cooperation with the Fairfax County Water Authority, was conceived initially as an opportunity to study extensively a water system that serves a large population (approximately 640,000) in a rapidly urbanizing area of Northern Virginia. Of special interest were viruses and several chemical constituents of raw and finished waters. Provisions were made to monitor the chemical constituents at varying frequencies during all seasons of two consecutive years. Virus monitoring, which was carried out during the first year, resulted in reported isolates from finished waters which evoked expressions of concern on several fronts concerning the reliability of presently available techniques for detecting viruses. As a result, virus monitoring was increased during the second year of the project, and in addition, provisions were made for the contracting party to be joined by EPA's virus-monitoring research group in a collaborative program of comparative sampling and methods evaluation. The entire project is truly unique in that, to date, more data concerning both raw and finished water concentrations of toxic substances and other constituents exist for FCWA's system than for any other in the United States.

The site for this project was especially attractive because there was an ongoing monitoring program (the Occoquan Watershed Monitoring Program, OWMP) to provide weekly water quality data for the Occoquan Reservoir and its tributaries which could be used in studies of possible correlations between raw and finished water quality. Too, during the period of this project an advanced waste treatment (AWT) facility was under construction which would effectively remove pollutants contributed to the reservoir by the discharge of approximately six million gallons per day of secondary treated sewage to Bull Run, one of the major tributaries. The Occoquan Project enabled an expansion of the monitoring effort of the OWMP to include organics, heavy metals, and viruses as part of the preconstruction data base. The AWT plant, owned and operated by the Upper Occoquan Sewage Authority went on-line in late June, 1978, approximately one year after the monitoring provided for by this contract was completed.

The Occoquan Project discussed in this report involved sampling the reservoir, a major tributary, and several sites in the distribution system. The report appears in two parts: Part I (issued September, 1979) discusses the data pertaining to trihalomethanes, pesticides, and metals, and Part II is concerned solely with the virus portion of this study.

EXECUTIVE SUMMARY

In 1975, a project to monitor viruses and chemical contaminants of water was begun in the Occoquan watershed and water-service area of the Fairfax County Water Authority (FCWA) located in northern Virginia near Washington, D. C. The virus-monitoring portion of the study was designed to supplement other virus monitoring being conducted by the FCWA and the Occoquan Watershed Monitoring Program (OWMP). The OWMP, ongoing since 1972, is administered by Virginia Polytechnic Institute and State University (VPI&SU) under the auspices of the Virginia State Water Control Board. Financing is supplied by political subdivisions that lie within the Occoquan watershed.

This report is the second of two parts and deals solely with the virus studies that were conducted over a 2-year period, ending May 1977. The first part (published September 1979, EPA-570/9-79-019) presented data concerning the chemical contaminants (heavy metals, pesticides, and trihalomethanes) of natural waters and FCWA's drinking water which it supplies to approximately 640,000 residents.

Occoquan-I

During the first year of the project (June 1975-May 1976, referred to as Occoquan-I), the emphasis was on virus monitoring: 1) in a major tributary stream - (two points - one upstream and one downstream of sewage treatment discharges on Bull Run), 2) from the source of FCWA's water supply (the Occoquan Reservoir), 3) the finished water at FCWA's New Lorton facility (one of three plants), and 4) at two points in the distribution system, one in Fairfax County and the other in Alexandria, Virginia. Two major objectives were 1) to determine the background levels of viruses in Bull Run and at the FCWA's raw-water intake as a means of assessing sewage discharge and urbanization effects and 2) to evaluate the capacity of conventional clarification-purification processes for removing viruses and the possible occurrence of viruses in the distribution system. The sampling events provided for by this contract were scheduled to supplement the biweekly schedule for virus-monitoring in raw and finished water already being carried on by FCWA and the OWMP.

During Occoquan-I, finished water at the three sites was monitored on 22 occasions, beginning in June 1975, and lasting through August 1975, under the provisions of the contract. The field procedures, which involved passing approximately 100 gallons of water through The Carborundum Company's Aquella$^{\text{tm}}$ Virus Concentrator, were carried out by OWMP personnel and field technicians employed by The Carborundum Company, who was VPI&SU's subcontractor. The samplings by FCWA and OWMP personnel in the ongoing program of virus monitoring also involved the Aquella$^{\text{tm}}$ concentrator and the schedule called for biweekly samples of both raw and finished water at FCWA's New Lorton facility. The concentration procedures were basically those listed as a tentative standard method in

14th edition of Standard Methods for the Examination of Water and Wastewater.

Once the field-concentration procedures were completed, the concentrates were frozen and shipped by air express to the University of New Hampshire's (UNH) Jackson Estuarine Laboratory (JEL) where they were assayed for enteric virus content by inoculating them onto animal-cell cultures--Buffalo Green Monkey (BGM) cells and African Green Monkey Primary Kidney Cells (PMK). The JEL was used exclusively for assay of field concentrates; to minimize the risk of contamination of field samples no laboratory-propagated virus strains were ever brought into the facility.

On four occasions out of the 22 sampling events during Occoquan-I, low numbers of a single enteric virus serotype (poliovirus type 1) were recovered from one of the finished water sites--twice at the treatment plant and once at each of the two distribution system locations. No viruses were reported in any of the 26 drinking water samples collected during the separate study by FCWA. On each occasion when virus recoveries were reported, the free chlorine residuals were greater than 1.0 mg/1 with probable contact times of several hours, and the pH was between 7.0 and 7.5, conditions normally regarded as adequate for virus inactivation. Coliforms were absent from samples taken during this period. Because of the unusual nature of these findings, the data, along with all field and laboratory procedures, as well as the water treatment processes, were closely reviewed. In addition, the isolates themselves underwent rigorous scrutiny at both UNH and the Center for Disease Control (CDC). In summary, the virus was characterized as a non-vaccine-like avirulent poliovirus type 1 strain. This extensive review failed to uncover any objective evidence to either refute or substantiate the findings. The Carborundum field sampling and procedures were reviewed, however, and were found to need improvement with respect to contamination prevention. The Carborundum Company did not have a program of routine personnel surveillance wherein throat- and rectal-swabs from the field technicians were routinely taken, so there was no way to determine if the isolates could have been the result of a chance contamination by a field team member who was actively shedding virus.

One would expect that viruses could be recovered more frequently from untreated natural waters than finished waters, especially if the natural waters were receiving effluents from municipal sewage-treatment plants, as was true of Bull Run, but such was not the case during the Occoquan Project. During Occoquan-I, there were 44 sampling events from natural waters; viruses were recovered on only three occasions. Six additional samples, processed by the OWMP during this period from FCWA's raw water intake, were also negative for viruses. The reasons for the low percentage of recoveries are not known. Either viruses were not there at all or were present in concentrations too low to be recovered by the standard procedure when approximately 100 gallons of water were processed. During Occoquan-I, the first clarifying filters, which could have retained viruses trapped in debris, were not eluted, a fact which might explain some of the failures to recover virus. However, in the OWMP virus sampling, the clarifying and adsorbing filters were eluted in processing their raw water samples, and no viruses were recovered from either filter type. The puzzling aspect of these data is that viruses were not found more often in untreated natural waters when, in the same time frame, viruses were recovered from finished drinking water samples on four occasions.

Occoquan-II Design

Environmental Monitoring--

Because the reported virus isolations during Occoquan-I were unexpected and apparently unusual, the EPA had an immediate interest in continuing and expanding the virus monitoring program. A second year's effort was designed which was more extensive than the first, and, in addition, provisions were made for the EPA Viral Diseases Group (Health Effects Research Laboratory; Cincinnati, Ohio) to periodically sample side-by-side with The Carborundum Company's field team. During these events, the final concentrates were to be subdivided, and each of the respective assay laboratories were to examine a portion of the other team's sample concentrates. In all, 13 such events were planned, including two at a local sewage treatment plant. The remaining 11 events included 9 at the 3 finished water sites and 2 at FCWA's raw water intake. In all, 55 individual sampling events from finished water and 19 from natural waters (Occoquan Reservoir at the raw water intake and Bull Run) were included in the environmental monitoring protion of Occoquan-II.

Prior to the beginning of Occoquan-II, The Carborundum Company critically reviewed its field procedures and made many modifications in order to eliminate as many opportunities as possible for a chance contamination of samples during execution of the field procedures. An enclosed truck, and later a van, was used to house the sampling equipment. The personnel worked inside the vehicle and disinfected it each day after use. In addition, a routine personnel surveillance program, involving rectal and throat swabs, was instituted to provide additional data in the event an isolate was reported in a sample.

Special Studies

Two special studies were planned for Occoquan-II. One consisted of a series of eight sampling events of water from FCWA water treatment plant filters immediately before, after, and during backwash. The rationale for this study was that clarification processes concentrate viruses in the floc, and because filters accumulate considerable quantities of the floc over a period of one or more days, the likelihood of recovering viruses should be greater at such a location during the times mentioned. The second study involved collecting samples of the unwashed filter-media (anthracite) and returning them to UNH where they were examined for the presence of viruses by the routine elution and subsequent assay procedures. Concentrates were divided for analysis by both EPA and Carborundum. No viruses were isolated from any of these samples.

Comparative Sampling and Analysis with Coded, Seeded Samples--

A final aspect of Occoquan-II, a truly unique one, was the design and execution of a rigorously controlled study designed to evaluate the sampling and analysis procedures used for virus monitoring by the EPA and The Carborundum Company. The plan included the involvement of an unbiased third party, the California State Health Department (CSHD) Viral and Rickettsial Diseases Laboratory, which, under separate contract with EPA, was to prepare blind, coded vials, some containing viruses and some containing only sterile diluting medium (blanks). The virus vials consisted of four different sets including polio 1 and polio 2 at four different titers i.e. 53, 250, 300 and 2.9 x 10^6 PFU (for 25 test runs) and sterile medium blanks (for 10 test runs).

A rather elaborate system for pasteurizing finished water was designed and constructed, and a plan was devised for the two field teams to sample on 35 separate occasions. On each occasion, the contents of a coded vial were added to the pasteurized test water, either through the thiosulfate reservoirs of the respective parties' concentrator system and thereby dosed into the water through the proportioner pump or added directly to the large, disinfected tank containing 250 gallons of the pasteurized test water. After 100 gallons of the water had been passed through their respective concentrators, the two parties followed their established protocols for eluting the filters and concentrating the eluates. Then each party divided its final concentrate into two, equal portions and returned them to FCWA for recoding. Each assay laboratory received one of the recoded samples prepared by the other field team and one prepared by their own team. For this portion of the study each lab used a common cell line (BGM, furnished by UNH) in order to minimize a possible source of error. After each party had completed all analyses and the results had been reported, the data were decoded, tabulated and sent to the principals in the project for analysis.

Occoquan-II Results

Environmental Monitoring and Special Studies--

On only one occasion during Occoquan-II was virus isolated out of 81 sampling events from drinking water (26 of these conducted by FCWA). On the date of the sole reported recovery, the rectal swab taken from the principal Carborundum field technician was positive for viruses: six poliovirus-1 and one Coxsackie B-4 were recovered from the specimen. The drinking water isolate was an avirulent, non-vaccine-like type 1 poliovirus, as were the poliovirus isolates from the rectal swab. Recovery of viruses from the rectal swab, while not definitive, did raise doubt regarding the validity of the environmental virus isolate from the distribution system.

In none of the 11 comparative sampling events from drinking water sites did either the EPA or The Carborundum Company report finding any viruses. Neither were any recovered during the special studies involving the filters and the backwash water. Both groups successfully recovered viruses in comparatively large numbers from two samplings of the sewage treatment plant effluent.

Viruses were recovered from very few natural water samples during Occoquan-II, as was true during Occoquan-I. Only three (one at each station) of 19 samples were positive for viruses, even though both the clarifying and adsorbing filters were eluted each time. An additional 26 samples were taken from FCWA's raw water intake for virus analysis as part of the OWMP, and no viruses were recovered from 52 subsamples (26 eluates each from the clarifying and adsorbing filters). As mentioned previously, the reasons for the low recoveries from natural waters of the Occoquan watershed during both years of the study are not known, but it does appear, based on the failure to find viruses in 143 of 149 subsamples from 95 different sampling events (including those by the OWMP) using state-of-the-art procedures, that viral contamination of FCWA's raw water source is not a significant problem, thereby failing to indicate a significant impact on virus levels from sewage treatment plant discharges or urbanizing activities.

Comparative Studies with Coded, Seeded Samples

The results of the comparative studies with coded, seeded, samples were quite revealing in several aspects. First, in the series of 25 experiments when the viruses were added to the thiosulfate reservoirs, the EPA procedure was more effective than the Carborundum procedure in concentrating the viruses from the water and in detecting them through the assay. The ranges of recoveries by the two groups during these studies were 36 to 59 percent for EPA and 0 to 20 percent for Carborundum. The percentages were calculated from inputs based on the initial seed sample titrations performed by CSHD. Second, in the series of experiments when viruses were added directly to the 250 gallons of pasteurized water, the performance of both procedures were about equal, but the efficiencies 0-15 percent and 0-18 percent for EPA and Carborundum, respectively, were notably lower than during the other tests. The reason is not known, but one possibility is that the viruses adsorbed to the walls of the tank or were otherwise removed before they reached the virus concentrators.

The third revealing aspect of the study concerned the matter of contamination. The EPA group reported on three occasions the presence of a type virus which had not been present in the coded input-vial. An analysis of their own records and procedures led the EPA group to conclude that inadequate equipment disinfection probably accounted for one of the three contaminations. No tenable hypothesis could be put forth to explain the other two contamination events. None of the 40 subsample analyses from the 10 experiments dosed with sterile medium (blanks) revealed the presence of viral contaminants.

The results of the comparative study underscore the need for additional research to devise procedures that will insure contamination-free results. They also demonstrate that a practical test for virus monitoring as a routine, water quality assessment procedure is not yet available.

This report was submitted in fulfillment of Contract No. 68-01-

3202 by the Research Division of VPI&SU and its subcontractor, The Carborundum Company, under the sponsorship of the EPA. This report covers the period from May 23, 1975 through May 31, 1977 and work was completed as of Feb. 1, 1978.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

BGM -- Buffalo Green Monkey
°C -- degrees Celsius
CPE -- Cytopathic effect
CSHD -- California State Health Department
EPA -- Environmental Protection Agency
FCWA -- Fairfax County Water Authority
ft -- feet
g -- gram(s)
gpm -- gallons per minute
HCl -- hydrochloric acid
HEK -- human embryonic kidney
JEL -- Jackson Estaurine Laboratory
km -- kilometer
ℓ -- liter
M -- Molar
μm -- micrometer
MCS -- membrane coating solution
mg/l -- milligrams per liter
MGD -- million gallons per day
ml -- milliliter
N -- Normal
OWMP -- Occoquan Watershed Monitoring Program
PEG -- polyethylene glycol
PFU -- plaque-forming unit(s)
PMK -- primary monkey kidney
RCT -- reproductive capacity temperature
STP -- sewage treatment plant
STS -- sodium thiosulfate
UNH -- University of New Hampshire
UOSA -- Upper Occoquan Sewage Authority

SYMBOLS

$AlCl_3$ -- aluminum chloride
Na_2CO_3 -- sodium carbonate
$Na_2S_2O_3$ -- sodium thiosulfate
$\bar{x}$ -- mean

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SECTION 1

INTRODUCTION

GENERAL DESCRIPTION OF THE STUDY

In June, 1975, an intensive monitoring effort, referred to as "The Occoquan Project," was begun in the Occoquan, Virginia, watershed and water-service area of the Fairfax County Water Authority (FCWA). The study, which began within six months after EPA's "eighty-city survey" (1) and the enactment of the Safe Drinking Water Act (PL 93-523), was designed to determine the concentration range and variability of a variety of health-related constituents of both the treated water from FCWA's distribution system, which serves approximately 640,000 residents of several northern Virginia communities, and the raw water source (the Occoquan Reservoir), including one of two of its major tributaries (Bull Run). The water constituents which were of interest were: a variety of pesticides; haloforms (principally the trihalomethanes); selected heavy metals, many of which are toxic to humans; and enteric viruses. The results concerning the chemical constituents have been presented in Part I of this report. (EPA-570/9-79-019, September 1979).

The Occoquan Project was conducted by Virginia Polytechnic Institute and State University (VPI&SU) and its subcontractor, The Carborundum Company, as a supplement to a larger, ongoing monitoring effort--the Occoquan Watershed Monitoring Program (OWMP)--that is funded by the several political jurisdictions that lie within the watershed and is conducted under the auspices of the Virginia State Water Control Board. That program, begun in 1972, is conducted by VPI&SU and provides weekly data concerning water quality at several points within the Occoquan Reservoir and along its major tributaries. The Occoquan Project provided for an expansion of the water-quality data base compiled by the OWMP before an advanced waste treatment facility, owned and operated by the Upper Occoquan Sewage Authority (UOSA), went on-line in June, 1978. The OWMP, in addition to its routine monitoring, has been the nucleus for several projects involving water-quality assessments, urban runoff, and other related subjects. Reports of several of these studies have appeared in the literature (2, 3, 4, 5).

This portion of the report contains a presentation and discussion of data concerning the virus-monitoring phases of the project during the first and second years of the project (hereafter referred to as Occoquan-I and Occoquan-II, respectively) and a special methods-development study in which the Viral Diseases Group of EPA's Health Effects Research Laboratory (HERL), Office of Research and Development, participated. The viruses of interest included three enteroviruses--polio, Echo, and Coxsackie--adenovirus, and reovirus. During the first year of the project, which began in late May, 1975, only routine monitoring for viruses from untreated and treated waters was involved. The purpose of this monitoring effort was to determine background virus levels in the raw water and to evaluate the capacity of conventional clarification-purification processes for removing viruses during the treatment of water prior to its distribution to consumers. The schedule was arranged so that the monitoring provided by the contract would supplement that being conducted routinely by FCWA and as part of the OWMP.

During the second year (Occoquan-II), the environmental monitoring effort was continued, but at less frequent intervals, and, during that year, The Carborundum Company's virus group and EPA's virus group participated in several side-by-side studies which involved monitoring of both raw and finished drinking water and, on one occasion, treated sewage. In addition, a rather elaborate, controlled study was designed and executed to evaluate the methods of virus sampling and analysis that were then being used by the two groups. That study, which will be described in considerable detail later in this report. involved the recovery of enteric viruses, whose concentrations and identities were not known by the participants, from large volumes of heat-sterilized water. Through this study, an evaluation of currently used virus-concentration and assay procedures was made possible.

The contract also provided for additional analyses during both years which would help characterize the water quality at the time of sampling. Included were: bacterial analyses [fecal and total coliforms plus standard plate counts (SPC)], temperature, pH, physical appearance, and a qualitative assessment of odor if any unusual condition was noted.

BACKGROUND INFORMATION

The Watershed

The watershed (area approximately 580 square miles), as shown in Figure 1, consists of portions of four counties: Fairfax, Fauquier, Loudoun, and Prince William. Two of the major tributaries join to form the Occoquan Reservoir, which was impounded in 1957. At full pool, the reservoir holds 9.8 billion gallons. The headwaters of the watershed are in forested and agricultural areas. Cedar Run and Broad Run drain areas (approximately 191 square miles) that are almost entirely rural and undeveloped from an urban perspective. On the other hand, Bull Run flows between and adjacent to Fairfax County and the Manassas area, two of the most rapidly urbanizing regions in the United States. Urban runoff and effluents from sewage treatment plants [about 5.4 million gallons per day (MGD)] flow into Bull Run and its tributaries, eventually reaching the reservoir. Construction of an advanced watewater treatment plant, under the auspices of the Upper Occoquan Sewage Authority (UOSA), was completed during 1978, greatly reducing one source of pollution in the reservoir. The urbanized areas, constituting approximately twenty percent of the total watershed, are mainly along Bull Run (bordering Manassas), along Flat Branch (draining Manassas to Bull Run), and along Big Rocky Run (a tributary to Cub Run)

Description of FCWA's Water Treatment Facilities

Fairfax County Water Authority has three interconnected treatment

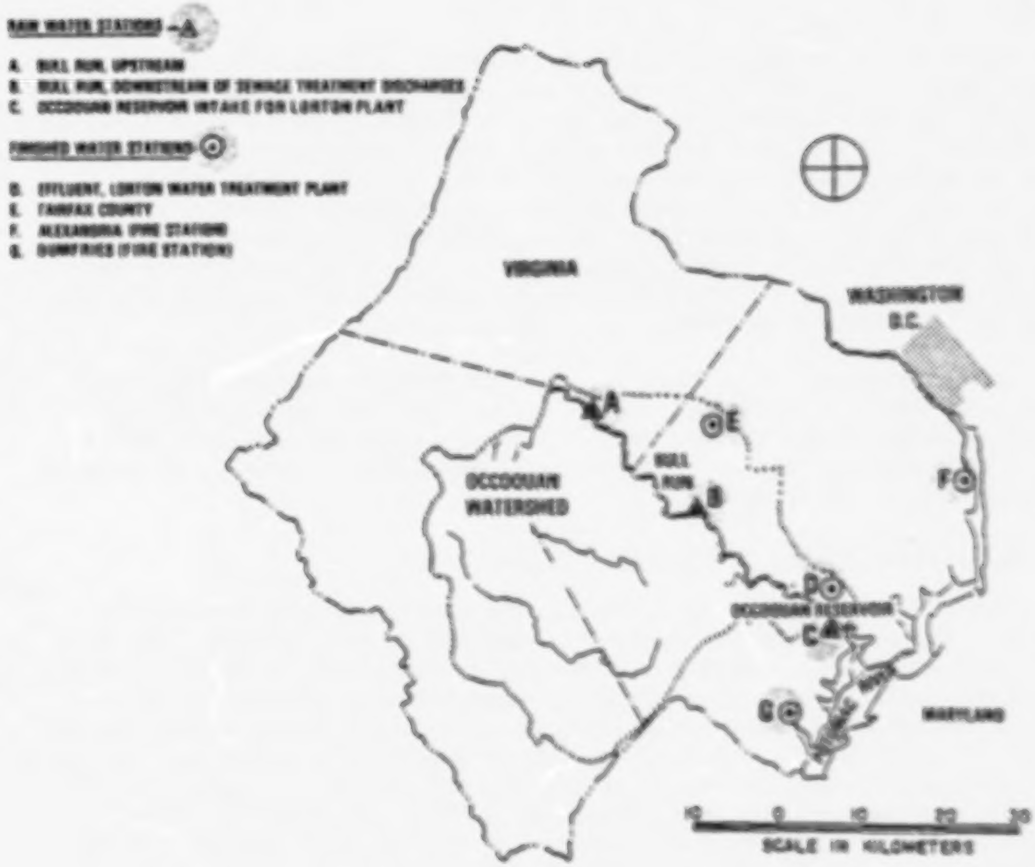
RAW WATER STATIONS -A
A. BULL RUN, UPSTREAM
B. BULL RUN, DOWNSTREAM OF SEWAGE TREATMENT DISCHARGES
C. OCCOQUAN RESERVOIR INTAKE FOR LORTON PLANT
FINISHED WATER STATIONS
D. EFFLUENT, LORTON WATER TREATMENT PLANT
E. FAIRFAX COUNTY
F. ALEXANDRIA (FIRE STATION)
G. DUMFRIES (FIRE STATION)
VIRGINIA
WASHINGTON D.C.
A
E
BULL RUN
B
F
OCCOQUAN WATERSHED
D
OCCOQUAN RESERVOIR
C
G
POTOMAC RIVER
MARYLAND
10
0
10
20
30
SCALE IN KILOMETERS

Figure 1. Map Showing the Raw and Finished Water Sampling Stations in the Occoquan Watershed and Fairfax County Water Authority's Distribution System.

plants: the Occoquan Plant, the Old Lorton Plant, and the New Lorton Plant. The latter was dedicated in 1973, and it employs conventional sedimentation-filtration units. The other two plants utilize aldridge sedimentation-filtration units. The design capacities of the three plants, in million gallons per day (MGD), are 20, 26.4, and 16, respectively, although the operating rates allowed by the Virginia State Health Department have been increased to permit production rates of 111.6 MGD with increased monitoring of turbidity in the finished water. The Occoquan Plant employs tube sedimentation and high-rate filtration at rates of 4-5 gallons per minute per square foot (gpm/ft^2). The other two plants employ conventional filtration at rates of 2 gpm/ft^2 and higher.

Water is taken from the Occoquan Reservoir through a 72-inch main. Pretreatment is usually chlorination only, but provisions are made for the addition of powdered activated carbon. Alum is the principal coagulant, but ferric salts are used occasionally. After flocculation, settling, and filtration, additional chlorine is added, and lime is added to increase the pH to approximately 7.5. Backwash water is sent to a reclamation basin, and from there the settled water is recycled to the head of the New Lorton Plant. Subsequent to the field sampling period covered by this project, the chlorination points in all three plants was moved in 1977-1978 to the settling basin discharge.

During the project period, the average finished-water production rate varied from 56.5 to 75.1 MGD (See Table 1). The approximate contribution by each of the three plants, as a percentage of the total during 1975-1976, were as follows: Occoquan, 45 percent; Old Lorton, 25 percent, and New Lorton, 30 percent.

Characteristics of Raw and Finished Water

Table 2 shows the average raw and finished-water characteristics recorded at the New Lorton facility during the project period. The data are shown as average quarterly values and include most of the routinely monitored constituents. Periodically, comprehensive analyses of the waters are performed, and concentrations of selected heavy metals, total organic carbon (TOC), carbon chloroform extract (CCE), chlorides, sulfates, phosphorus, nitrates, and total solids are determined. In summer, 1977, a monitoring program for trihalomethanes (THM's) was begun by FCWA personnel to evaluate the effectiveness of varying treatment processes in reducing finished-water THM concentrations.

As can be seen from Table 2, the raw water is low in alkalinity (and dissolved solids, as reflected by the low specific conductivity), and addition of coagulants reduces the pH to less than 7.0, necessitating lime addition to increase the buffering capacity of the finished water. Powdered activated carbon is added periodically to control tastes and odors generated primarily by algae in the reservoir. Copper sulfate is applied at several points in the reservoir, usually from May through October. In 1975, 78.7 tons were applied, 69 percent of which was in July and August. In 1976, only 52.7 tons were applied, approximately 52 percent being added in July and August. Since 1971, the Authority has successfully improved raw water quality at the intake by forcing air through perforated, plastic pipes that lay on the bottom of the reservoir extending from the dam for several hundred feet upstream.

TABLE 1. AVERAGES AND RANGES OF FINISHED WATER PRODUCTION RATES AT FAIRFAX COUNTY WATER AUTHORITY DURING PROJECT PERIOD

Time Period		Finished Water Production Rate, MGD[a]	
		Average	Range
June-Sept.	1975	65.9	52.5 - 89.5
Oct.-Dec.	1975	56.5	51.6 - 63.2
Jan.-March	1976	57.7	53.0 - 63.5
April-June	1976	72.5	60.0 - 99.0
July-Sept.	1976	75.1	60.7 - 97.0
Oct.-Dec.	1976	63.7	56.7 - 74.2
Jan.-March	1977	63.6	56.2 - 74.3
April-May	1977	72.4	61.3 - 98.4

[a] MGD=million gallons per day. To convert to cubic meters per day, multiply values shown by 0.3785.

TABLE 2. AVERAGE CHARACTERISTICS OF RAW AND FINISHED WATER AT THE FAIRFAX COUNTY WATER AUTHORITY'S NEW LORTON TREATMENT FACILITY DURING PROJECT PERIOD, MAY 1975–MAY 1977

Time Period		Turbidity Units		Alkalinity, mg/l		pH Units		Color Units		Hardness, mg/l		Mn^{++}, mg/l		Specific Conductance, µmhos/cm		Total Chlorine, mg/l
		Raw	Fin.	Raw	Fin.	Raw	Fin.	Raw	Fin.	Raw	Fin.	Raw	Fin.	Raw	Fin.	Fin.
May-June	1975	2.7	0.10	35	40	7.2	7.7	27	3	46	67	0.10	0	-	-	1.9
July-Sept.	1975	8.2	0.12	34	41	6.8	7.3	58	5	42	72	0.19	0.01	96	151	2.6
Oct.-Dec.	1975	8.7	0.12	30	36	6.9	7.5	53	3	42	70	0.09	0	112	160	2.7
Jan.-March	1976	19.1	0.20	25	28	7.1	7.6	55	5	43	64	0.07	0	104	147	2.4
April-June	1976	5.7	0.10	30	37	6.9	7.5	50	3	45	71	0.13	0	104	162	2.9
July-Sept.	1976	1.9	0.07	44	50	7.0	7.5	25	5	52	75	0.17	0	134	192	3.3
Oct.-Dec.	1976	18.2	0.15	27	32	7.0	7.5	79	5	42	72	0.17	0	84	122	2.9
Jan.-March	1977	8.4	0.27	33	37	7.0	7.6	49	11	53	91	0.14	0.01	121	174	2.5
April-May[a]	1977	5.8	0.05	29	31	7.0	7.4	45	10	44	75	0.10	0	-	-	2.6

[a] Data through May 19, 1977.

SAMPLING SITES

During the first year of the study, there were six sampling sites: two on Bull Run upstream and downstream of major discharges of treated sewage and urban runoff, two at the FCWA's New Lorton Water Treatment Facility (both raw and finished waters) located near the reservoir high dam, and two at distant points in the distribution system. During the second year, the Bull Run sites were sampled only once, and an additional site in the distribution system was added to the sampling schedule. A detailed description of these sites is given in Table 3.

The particular location for Site F was selected originally because an open-storage reservoir is located between it and the water treatment plant. During the day, when the demand is low, the reservoir fills. Between 4:00 p.m. and midnight, when the demand is high, the reservoir is used to supplement the flow from the FCWA plant. However, during this study, most all samples were taken at times when there was no contribution from the open reservoir.

Figure 2 provides a schematic of the virus sampling stations selected (also shown on the map in Figure 1) and the possible expected results. First, Site A above the sewage treatment discharges was selected to provide low background data. Next, Site B was selected downstream of sewage outfalls to reflect possibly increased virus levels. The last raw water site (C) was selected to show the virus levels presented for treatment by the New Lorton Treatment Plant; virus levels here would likely be lower than at Site B. Finally, four treated water sites were selected; one of the New Lorton Plant effluent (D) and three at distant points in the distribution systems (E, F, G). Site G was the most distant from the treatment plant. Interestingly, the study results failed to confirm many of the original expectations.

Three additional sites were selected for short-term studies in which both the virus groups from EPA and The Carborundum Company participated. These sites were: 1) the effluent from the secondary clarifier and the effluent from the subsequent storage lagoon (approximately 30-days detention) at a local sewage treatment plant (Northside Sewage Treatment Plant, a trickling-filter operation); 2) the open-storage reservoir used by the Virginia American Water Company in Alexandria, Virginia, to store treated water from FCWA to augment flow during peak demand periods; and 3) below the filter gallery at FCWA's New Lorton facility. Water was sampled immediately preceding backwash of a filter, again during the backwash,, itself, and, finally, immediately after the backwashing cycle was completed.

The sewage treatment plant was selected because it was expected that the virus concentration would be quite high, and recoveries by both sampling parties could be virtually assured. The open-reservoir in Alexandria was sampled to determine if such a method of water storage might possibly provide an opportunity for chance contamination of potable water. The studies involving the filter backwashing process were conducted to determine if the filter *itself might store viruses or if the* disruption of the filter by backwashing might be conducive to virus breakthrough immediately after the backwashing was complete.

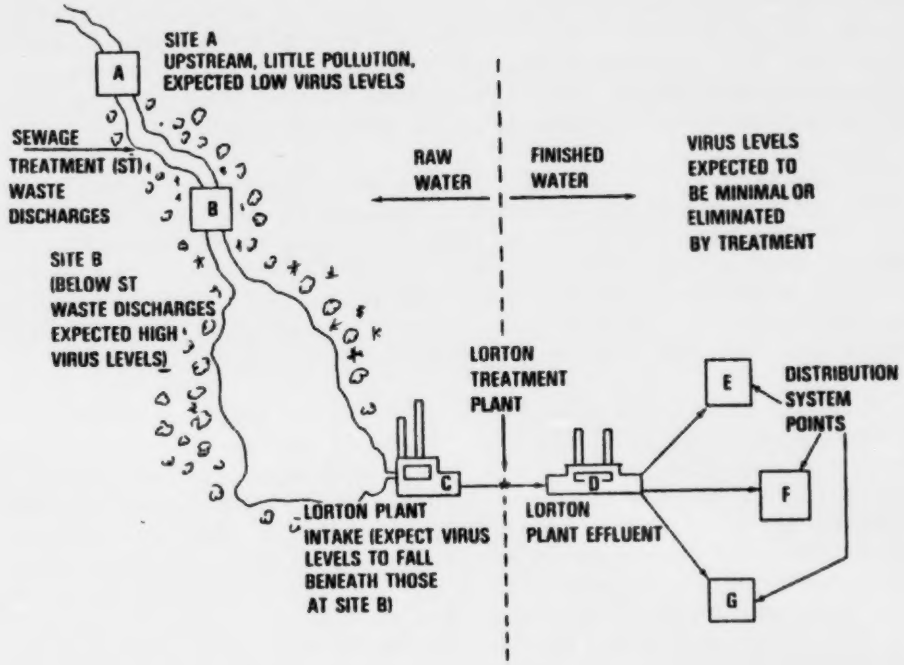
SITE A
UPSTREAM, LITTLE POLLUTION,
EXPECTED LOW VIRUS LEVELS
A
SEWAGE
TREATMENT (ST)
WASTE
DISCHARGES
B
RAW
WATER
FINISHED
WATER
VIRUS LEVELS
EXPECTED TO
BE MINIMAL OR
ELIMINATED
BY TREATMENT
SITE B
(BELOW ST
WASTE DISCHARGES
EXPECTED HIGH
VIRUS LEVELS)
LORTON
TREATMENT
PLANT
E
DISTRIBUTION
SYSTEM
POINTS
C
D
F
LORTON PLANT
INTAKE (EXPECT VIRUS
LEVELS TO FALL
BENEATH THOSE
AT SITE B)
LORTON
PLANT EFFLUENT
G

Figure 2. Schematic of Virus Sampling, Occoquan Study. 1975 - 1977.

TABLE 3. OCCOQUAN PROJECT SAMPLING SITE LOCATIONS AND DESCRIPTIONS *

Site	Location	Conditions
*A	On Bull Run, 23 miles upstream of the confluence of Bull Run and Occoquan Creek. Site is designated "Catharpin" and is at a point where Rt. 705 crosses Bull Run.	Forested and agricultural areas. Represents conditions upstream of major sewage treatment plants and urban drainage.
B	On Bull Run, 3.1 miles below confluence with Cub Run and 2.3 miles below the discharge of the last of 11 sewage treatment plants. The site is 14 miles below Site A, approximately 9 miles above the confluence of Bull Run and Occoquan Creek.	Average flow approximately 75 cfs. Bull Run at this point has received approximately 5.4 MGD of treated, chlorinated sewage from 11 plants (6 from Prince William Co. and 5 from Fairfax Co.) as well as urban and agricultural drainage.
C	Approximately 1000 feet below the Occoquan Reservoir dam from the raw water main to the Fairfax County Water Authority's (FCWA) water treatment plant. Samples were taken from inside the "carbon house". The site is approximately 18 miles from Site B and 9 miles from confluence of Bull Run and Occoquan Creek.	Raw water as received by the Fairfax Co. Water Authority's (FCWA) three treatment plants, combined capacity approximately 60 MGD.
D	Pump Station No. 2 of the Lorton High Service Plant at FCWA water treatment plant.	A mixture of finished waters from the three treatment plants of the FCWA.
**E	Within the distribution system in Fairfax County at the Fairfax County Storage Yard on Hwy 29 near County Road 645.	Finished water approximately 10 hours distant from the FCWA water treatment plants at average flow.
F	The Prince Street Fire Station in Alexandria, Va. At times, water from an open storage reservoir is rechlorinated and used to supplement flow from FCWA.	Finished water approximately 8 hours distant from FCWA water treatment plants at average flow. The system belongs to the Virginia American Water Company.
G	The Dumfries-Triangle Volunteer Fire Department, Inc. 18329 Jefferson Davis Highway.	Finished water approximately 13 hours distant from FCWA water treatment plants at average flow. The water going to this site is derived totally from the Occoquan Treatment Plant.

*Site A is sampled routinely as part of the OWMP. The other sites listed are separate from those sampled by OWMP personnel.

**This site was sold in February, 1978, and is no longer under FCWA's control.

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SECTION 2

CONCLUSIONS

The following conclusions are based on the results of the virus studies conducted during the Occoquan Project, June, 1975 through May, 1977.

1. During the first year of this project (Occoquan-I), finished drinking water was monitored for viruses on 22 occasions during the summer of 1975 under the provisions of this contract and an additional 26 times on a bi-weekly basis during 1975, by the Fairfax County Water Authority (FCWA) in conjunction with the Occoquan Watershed Program (OWMP - not part of this contract). Recoveries of single, non-vaccine-like, avirulent poliovirus isolates (type 1) were reported on four occasions, which represents an inordinately high percentage of the total samplings based on current knowledge of water treatment efficiency for virus inactivation. This rate of recovery was significantly higher than for the untreated natural waters, an anomalous result (see Figure 2 in the Introduction Section). A review of the field data, along with several subsequent investigations into the nature of the virus isolates, the laboratory procedures, and treatment plant operations, failed to uncover any objective evidence to either refute or firmly substantiate the conclusion that viral isolates originated from the finished water. No personnel surveillance program was conducted during the first year's effort, however, and the lack of such data limited the extent to which the possible sources of the isolates could be explored.

2. During the second year (Occoquan-II) improved field techniques were instituted, a program of personnel surveillance was established, and provisions were made for comparative sampling and analysis with the Cincinnati-based, EPA Viral Diseases Group (Health Effects Research Laboratory). On one occasion a virus isolate was reported out of 81 sampling events from finished water (55 provided by the contract, 26 by FCWA). The single isolate (also a non-vaccine-like, avirulent poliovirus, type 1) was recovered on a day when the principal field technician's rectal swab was positive for virus (6 type 1 poliovirus and 1 coxsackie B-4 virus), a fact which casts doubt on the actual source of the finished-water isolate.

3. Whereas one would expect to recover viruses quite frequently from natural, untreated waters contaminated by sewage, the number of occasions when that occurred were quite small. During Occoquan-1, there were 44 sampling events from natural waters; viruses were recovered only on three occasions, once at each natural water station (upstream, downstream of sewage treatment plant discharges and at the FCWA's raw water intake). During Occoquan-II, only three of 19 samples were positive (again, one at each natural water station), even though both

the clarifying filters and the adsorbing filters (parts of the virus concentrator) were eluted. An additional 38 samples were taken for virus analysis at FCWA's raw water intake as part of the ongoing OWMP with no viruses being recovered from 76 subsamples (adsorbing and clarifying filter eluates). The reasons for the low recoveries of viruses from natural waters, assuming they were present, lie in the limitations of the current environmental virus recovery procedures. Taking these limitations into consideration, the results (6 recoveries, 149 concentrates from 95 sampling events) do, however, appear to show no significant virus contamination of the FCWA's raw water source, thereby failing to indicate a significant impact on virus levels from sewage treatment plant discharges or urbanizing activities.

4. During Occoquan-II, both laboratory groups demonstrated their ability to recover and analyze viruses in limited, joint, environmental sampling from a sewage treatment plant effluent. In eleven joint-sampling events of finished drinking water, no viruses were found.

5. Attempts to recover viruses from water immediately before, after, and during the backwash of water treatment plant filters were unsuccessful on eight occasions. No viruses were found in the final sample concentrates, half of which were assayed by each participant in the comparative program, though one would expect that sampling at such a location and at those times would provide the greatest opportunity for recovery of viruses which might have persisted during the water-treatment process.

6. A carefully controlled, large-scale, double-blind experimental study was conducted in order to evaluate both the efficiency and reliability of currently used methods for recovering and detecting small numbers of viruses from large volumes of potable water. The results showed the following:

a. When viruses were added to the thiosulfate reservoir (part of the concentrator equipment) the EPA Viral Diseases Group's performance (based on the percent recoveries reported by both parties that participated in the study) was superior, both in concentrating the viruses from water and in detecting them during laboratory assays of the samples. The ranges of recoveries reported by the two groups (EPA and The Carborundum Company) were 36-59 percent and 0-20 percent, respectively, based on the original titer levels determined by the independent laboratory which supplied the viruses.

b. When viruses were added directly to a large tank containing 250 gallons of water (a situation more closely approximating conditions in a community water supply), the performance of both groups was about equal (0-15 percent and 0-18 percent for EPA and The Carborundum Company, respectively). These levels, much lower than the thiosulfate reservoir recovery efficiencies, raise basic questions about accepted practices of testing for virus recovery efficiencies and about the effective recovery efficiencies being obtained in virus sampling of clean finished drinking waters.

7. There were substantial, detectable differences in the efficiencies

of both the virus-concentration and virus-assay procedures employed by two, independent laboratories that were following currently accepted techniques for virus recovery from drinking water. These findings, together with the low level of tank test recoveries, point up the need for more developmental research into the most desirable procedures involved with virus monitoring, from the collection of samples to shipping and handling procedures and, finally, for the assay itself. Current methods, at best, are conservative in their potential for reliably detecting the presence of low levels of viruses (e.g. at levels of one in 100 gallons, the standard suggested by some).

8. Whereas contamination was suspected to have been responsible for the reported virus isolates from drinking water during Occoquan-I and II, the comparative study described in Conclusion 6 demonstrated beyond all doubt that the possibility of contamination is an ever-present danger whenever monitoring for low-level virus contamination of drinking water is attempted. The EPA virus-research group reported contaminants in three of the 70 subsamples which they analyzed. Among the several plausible explanations, the most likely seemed to point to improper equipment sterilization in one instance; no tenable hypothesis could be put forth to explain the other two contamination events. None of 40 subsample analyses from 10 experiments dosed with sterile medium (blanks) revealed the presence of viral contaminants. Control tests were routinely conducted in both assay laboratories to guard against reports of false positives in water samples. In the light of all the expertise, concern, and care put into this project, the report of contamination serves to emphasize the complexities involved in effective virus monitoring. The results of this study underscore the need for additional research to devise procedures that will insure contamination-free results. They also demonstrate that a practical test for virus monitoring as a routine water quality assessment procedure is not yet available.

SECTION 3

RECOMMENDATIONS

1. The results of this study indicate that the present day, virus monitoring technology, while extremely well developed compared to that of a decade ago, still is not sufficiently precise to permit routine surveillance of drinking water supplies for the reliable detection of one or two viruses in a large volume of water, say 100 gallons. Therefore, further methods-development research is needed under conditions that simulate actual environmental conditions as nearly as possible.

2. While those concerned with environmental monitoring for viruses have always been aware of the need for attention to contamination prevention during sampling, the results of this study have clearly shown that contamination can occur even when stringent precautions are being taken to avoid it. Therefore, it is recommended 1) that a thorough review of all existing procedures, both field and laboratory, be undertaken to identify those operations where even the remotest possibility of contamination exists and then, 2) that a detailed protocol for contamination control and quality assurance be developed, tested and established for inclusion in any future standard method.

3. As initially proposed, this project envisaged two parts - before installation of the Upper Occoquan Advanced Wastewater Treatment (AWT) Plant and after the plant was put in operation. In view of the low levels of virus found in raw waters and the expected higher efficiency of virus removal in the AWT plant, as compared to the secondary plants, further study in an "after installation" mode would not appear to be fruitful. However vigilance must be maintained regarding the possible virus contamination of natural waters by any waste discharge.

4. Until there is further development of procedures to improve virus sampling and analysis, including strict protocols for contamination prevention during sampling, it is recommended that current procedures still be applied to research investigations where it is felt that virus contamination of untreated or treated drinking water may be a problem.

SECTION 4

MATERIALS AND METHODS

A project such as this one would not have been possible fifteen years ago before the technology existed to make possible the recovery of viruses when only a few were present in large volumes of water. The techniques, which will be described in considerable detail, were essential because viruses do not exist in large enough numbers in most waters to be detectable by direct assay. There first must be a concentration step.

To give some idea of how dispersed viruses in nature are, Sproul (1) has estimated that surface waters in the midwestern United States contain only approximately three "plaque-forming units" (PFU's) per gallon. (The PFU is used to designate one infective unit, whether one or several viruses in a cluster, which causes a single lesion to appear in a laboratory-cultured animal-cell sheet.) He also estimated that a properly operated water treatment plant might reduce this level of viruses to one PFU per thirty million gallons.

The contamination of public water supplies by viruses which are of public health significance most often occurs because human sewage, whether treated or untreated, has been discharged to those supplies. There are more than one hundred known human enteric viruses, and because they cannot survive outside living cells, their numbers, after they are discharged to the environment, invariably decrease. Because they are present in such low numbers, the task of recovering them is quite difficult. Shaffer _et al_. (2) have put the problem in perspective. They calculated that one virus in one gallon of water is equivalent to only one part in 10^{20} parts, whereas, by contrast, one part per billion (ppb), which is considered to be a microquantity in chemical analyses, is one part in 10^{9} parts.

The importance of a human ingesting a single virus particle capable of infecting a cell is difficult to ascertain. Plotkin and Katz (3) have postulated that the minimum infective dose, i.e. that which is required to initiate infection, is only one PFU. Others apparently agree, though the subject is debatable (4). Melnick (5) has proposed that any future virus standard for drinking water should be "one detectable infectious unit per one hundred gallons."

While the technology which will permit detection of viruses at such low levels is reasonably well advanced, only the larger water utilities in this country would be able to undertake the task of virus monitoring, both because the cost is extremely high and the technology is quite complex. Hill _et al_. (6) have speculated that an alternative microbial indicator system or minimal treatment criteria may be required in the future to assure that drinking water is "virologically safe."

The methods used by both the EPA Viral Diseases Group and The Carborundum Company's personnel to concentrate viruses from large volumes of water were approximately equivalent to those which have been proposed as tentative methods in the fourteenth edition of Standard Methods (7). The specific procedures followed by both groups are presented in detail in the following:

SAMPLING AND ASSAY PROCEDURES

Before the specific details of methods used by both participants in this project are presented, a general overview of the basic procedures involved in virus concentration and assay would be useful. While a number of different concentration procedures have been developed with varying degrees of success, (see Hill _et al._, reference 8, for an excellent review) only that one which _is the_ tentative standard method will be discussed. Several modifications to this basic procedure have been suggested by others (e.g. Sobsey, reference 9) since this project was conducted, but both participants in the Occoquan Project followed approximately the same procedures which will be described.

Basic Virus Concentration Procedures

Figure 3, adapted from a presentation by Shaffer _et al_. (2), shows in rudimentary detail the sequence followed in recovering viruses from water. The discussion given by Shaffer _et al_. is summarized as follows: First, the water is filtered, if necessary, to remove interfering solids. Then chemicals are added to adjust the pH and cation concentration to enhance viral adsorption on the next filter in the system. Next, the submicron filters holding the adsorbed viruses are treated with an alkaline solution which reverses the polarity of the virus particle, permitting it to be eluted from the surface where it had been bound. The eluted virus is further reconcentrated (by one of four methods which will not be discussed here) to reduce the final volume to one which is easily handled in laboratory assay procedures. Further steps include neutralizing the reconcentrated eluate, adjusting the salt concentration to promote virus stability, and adding a protective colloid. Finally, the solution is sterilized (e.g. by filtration through submicron membrane filters or treatment with ether) to remove bacterial and other microbial contaminants. The final concentrate may be frozen, if necessary, until the actual assay procedure in the laboratory is begun.

Basic Viral Assay Procedures

Because viruses are obligate, intracellular parasites, the only way to replicate them is to provide them with a host cell system of some type. Live animals, chick embryos, or cell cultures are suitable hosts, though the most common procedure for assaying waterborne viruses involves cell culture inoculation.

This procedure, in brief, involves the following basic steps: Tissues from a suitable donor organ (e.g. monkey kidney) are treated with enzymes to separate the individual cells. A suspension of these

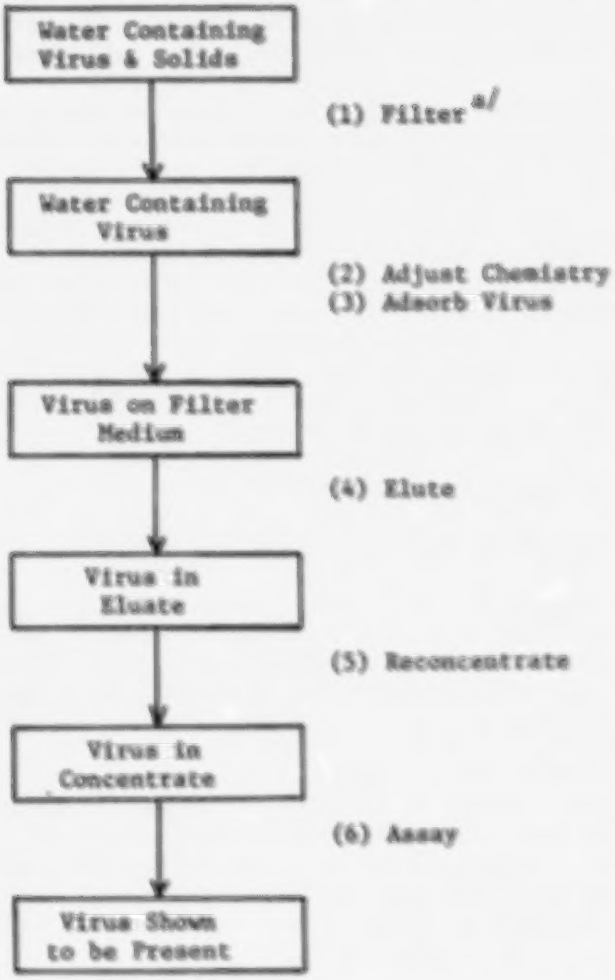

Water Containing Virus & Solids
(1) Filter[a]
Water Containing Virus
(2) Adjust Chemistry
(3) Adsorb Virus
Virus on Filter Medium
(4) Elute
Virus in Eluate
(5) Reconcentrate
Virus in Concentrate
(6) Assay
Virus Shown to be Present

Figure 3. Sequence of Steps to Recover and Demonstrate the Presence of Virus. (Reference 2)

[a] Not all virus sampling trains include a clarifying filter. The Carborundum equipment did, but the EPA's did not.

cells is then placed in a small, glass bottle with a nutrient medium and incubated. The cells will attach to the glass and begin to reproduce, eventually forming a monolayer cell sheet. Once the cell sheet has formed, the concentrate containing the viruses is placed in the bottle and gently dispersed over the cell layer. After a suitable time, during which the viruses "infect" tho host cells, the liquid is drained and replaced with a solid or semi-solid agar-based medium which covers the host cells and prevents newly formed viruses from an originally infected cell from spreading unchecked over the entire surface of the cell sheet. A vital stain is also added. In time, viral activity can be determined either by microscopically detectable changes in cell morphology or by macroscopic "plaques" in the cell sheet.

Plaques are formed when viruses produced inside the initially infected cells spread to the surrounding cells, infecting them and repeating the process. After a time, the area in which adjacent cells have been killed is visible, appearing as a clear area in the cell monolayer which does not take up the vital stain. Each plaque is considered to have arisen from a single infective virus, though clumps of virus may behave as a single unit. To ensure that plaques were indeed viral induced and not the result of some other cytopathic effect (CPE), such as chemical toxicity, the plaques can be "picked" with an inoculating needle and the collected material is passed onto new cell cultures. If the plaques form again, the investigator is assured that they were caused by a virus.

The Carborundum Company's Procedure

Concentration Equipment and Field Technique--

The Carborundum Aquella^{tm} virus concentrator was used throughout this program for field processing of water samples. During Occoquan-I, the concentrator was transported in a van by the field team to each sampling site, and the concentration procedures were carried out with the equipment exposed to the environment. During Occoquan-II, the concentrator was housed inside a truck (later in a large van), and all procedures were carried out inside the truck. The enclosed mobile vehicles provided a cleaner, more reproducible environment than when the concentrator was being used outside the vehicle at the various sampling sites. A local power source was used when available but a gasoline-powered generator was used in remote sites. The sample concentration procedures, discussed in the following paragraphs, are basically those found in Standard Methods (7).

Sterilization of equipment--The Aquella^{tm} concentrator was sterilized with dilute hydrochloric acid (HCL). All pumps, sampling lines, filter holders, flow-measuring devices, and filter holders with the appropriate filters in place were exposed to the dilute acid (pH 0.5 or lower) for at least thirty minutes. The sterilizing solution was pumped through the adsorbing filter system first, then through the remainder of the apparatus. Care was taken to ensure that the effluent from the clarifying system did not contact the adsorbing filter media. After the thirty-minute contact period, the concentrator was rinsed with the water to be sampled, and rinsing was continued until the influent and effluent pH were essentially the same. As before, the effluent from the clarifiers was not permitted to pass through the adsorbing filters. The exterior of the filter holders, the tubing, and the quick-connect fittings were similarly treated daily to minimize the chance of contamination.

Chemical additives--Routinely, two solutions were prepared and simultaneously added to the clarified sample water via Johanson proportioning pumps. The first, a solution of 0.03 molar (M) aluminum chloride ($AlCl_3$), was acidified with HCl so that when it was added to the sample water at a rate of one part solution to twenty parts sample, the final concentration of $AlCl_3$ was 0.0015 M and the pH was between 3.5 and 4.0. The second solution was sodium thiosulfate ($Na_2S_2O_3$) [6.74 grams (g) per liter (ℓ) for each milligram per liter (mg/ℓ) of chlorine present in the sample stream] was injected into the sample water stream with a second Johanson pump at a dilution rate of 1:100. Dechlorination of the sample stream was verified by testing with either an orthotolidine or DPD test kit.

Clarifying filters--When the sample turbidity was high, as when stream water or reservoir water were being sampled, clarification of the influent was achieved by passing it through one or more fiber-wound cartridge filters. These filters, 019R105 and 03R105 (Commercial Filter Division; The Carborundum Co.; Lebanon, Indiana) were, respectively, 1.0 and 10.0 micrometer (μm) nominal porosity and constructed of Orlon wound on stainless-steel supports.

Adsorbing media--Viruses were adsorbed from the acidified water by passing it first through a wound-fiber, glass filter (K27R105), then through two flat filters (1.0 and 0.45 μm porosity) which were made from epoxy-bonded, asbestos and fiberglass materials (Cox Instruments, Detroit, Michigan).

Saline rinse--After the desired volume of clarified (if needed), acidified water containing $AlCl_3$ was passed through the adsorbing filters, a filter-rinse with saline at pH 3.5 was provided to remove any residual chemicals which might otherwise find their way into the eluate and adversely affect reconcentration. Typically, two 1.0 ℓ portions were forced through the filters by air pressure and discharged to waste.

Glycine elution--After the saline rinses were completed 1.0 ℓ of a glycine solution, pH 11.5, was forced into the filter holder, filling it completely, and then through it at a rate slow enough to ensure a contact time between the glycine and filters of one to two minutes. During Occoquan-I, the glycine concentration was 0.55 M, but it was increased to 0.10 M for Occoquan-II studies.

The elution step was repeated at least twice. The pH of the last effluent from the adsorbing filters was checked to see if it was between pH 11.0 and 11.5. If it was, the elution was considered completed; if not, additional portions of alkaline glycine were passed through the filters. At times, these procedures resulted in large volumes of eluate, but the elution procedure was not considered to be complete until the final pH was 11.0-11.5.

Neutralization--The eluates were neutralized with 0.05 M (later, 0.1 M) glycine, pH 2. The neutralization was performed quite quickly after elution of the adsorbing filters. These neutralized eluates can be stored for short periods at 0°-5°C.

Reconcentration--During Occoquan-I, the neutralized eluate was reacidifed and passed through a small Cox filter for readsorption and subsequent elution with a much smaller volume of alkaline glycine. The final volume was often small enough that the neutralized eluate would be placed directly on cell sheets for assay without further reconcentration. During Occoquan-II, the procedure was changed to a "gel reconcentration" procedure. This procedure is explained as follows:

The first eluate was adjusted to approximately pH 7 with the acidic glycine solution, and sufficient $AlCl_3$ solution was added to achieve a final concentration of 0.003 M. During this step, the pH may decrease to 4.0-5.0 and a floc may form. The mixture was stirred and sufficient 1.0 M sodium carbonate (Na_2CO_3) was added slowly and constantly until the solution pH stabilized at 7.2 for several minutes. Then the floc was allowed to settle for thirty minutes. If the floc settled well, the supernatant was decanted and wasted. Then, the remaining suspension was centrifuged for ten minutes to further concentrate the floc. When the floc did not settle well, the entire eluate volume was centrifuged.

Final concentration--When the gel reconcentration step was included, the final step involved combining the pellets from the individual centrifuge tubes with sufficient 0.10 M glycine, pH 11.5, to raise the pH of the mixture to 9.5. Then, this suspension was centrifuged for ten minutes. The centrifugate (liquid above the pellets) was recovered and neutralized to pH 7.5 by addition of pH 2.0 0.10 M glycine. Then 10 percent (by volume) of heat-inactivated fetal calf serum was added, and the sample was quickly frozen and stored in a freezer until it was shipped to the assay laboratory.

Shipment, receipt, and storage--All concentrates were shipped on dry ice (-78°C) in polycarbonate centrifuge tubes (50-ml, Oak Ridge type) enclosed in styrene shipping containers and packaged in a styrofoam container wrapped in a heavy-cardboard carton (Polyfoam Packers Corp., Chicago). The common carrier from Washington National Airport was the Airborne Freight Company which transferred packages to the C&J Limousine Service, located at Boston Metropolitan Airport, who delivered them to the University of New Hampshire (UNH) at Durham. Care was taken to ensure that the samples were not stored for a weekend at the airport or lost because shipping information was lacking. Telephone contact was made with personnel at The Carborundum Co. in Niagara Falls, N. Y. at the time of shipping so that all parties could be alerted. All federal and airline regulations were observed.

Upon receipt at UNH, each sample was assigned a number and placed in a Revco freezer at -70°C or lower until the assays could be performed.

Viral Assay Procedures--

Samples were assayed for viruses in the Jackson Estuarine Laboratory (JEL), Durham, New Hampshire. This facility was reserved entirely for the enumeration and identification of viruses in environmental samples. No research was conducted there, so no laboratory viruses were ever permitted into the facility to reduce the likelihood of contamination. The JEL is a portion of a larger laboratory involved with non-virus UNH research and is several miles from UNH's main campus where the virus research laboratory of Dr. T. G. Metcalf is located. An overview of the procedures to be discussed in the following sections is presented in Figure 4.

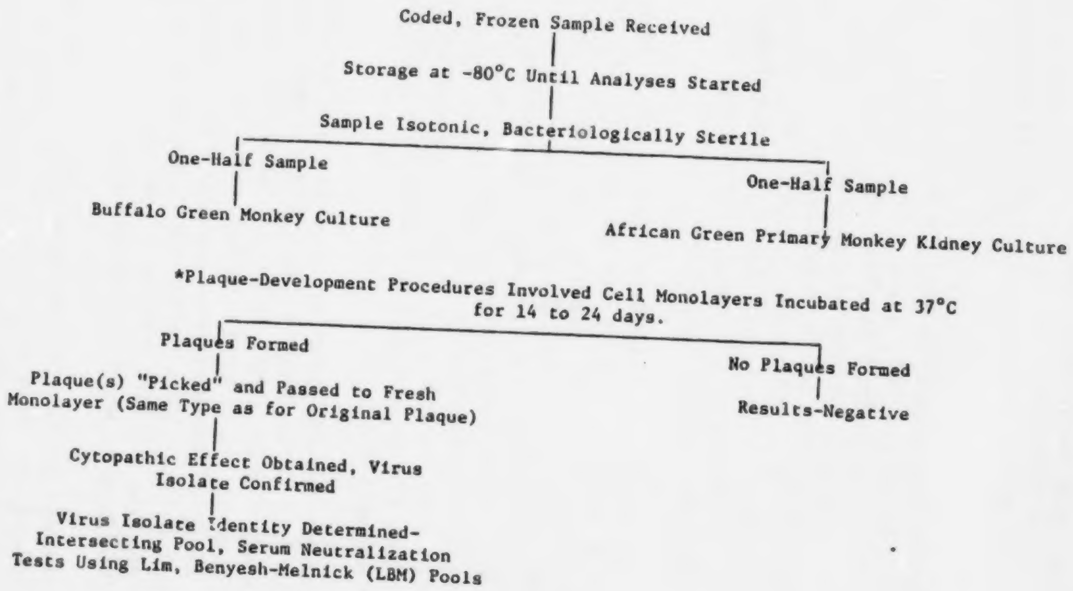
Reconcentration--Whenever the volume of the field-concentrates was too great to be placed directly on the cell lines, an additional concentration by "hydroextraction" was performed at JEL. This procedure actually involves dialysis. The concentrate was placed in a dialysis membrane (Union Carbide), covered with solid polyethylene glycol (PEG 20M, Union Carbide), and placed in a refrigerator until the desired volume reduction was accomplished. The hydroextracted concentrate was recovered from the dialysis tubing, and the tubing itself was rinsed with a three-percent beef extract (DIFCO) solution.

Virus enumeration and identification--Virus concentrates were treated with diethyl ether (10-20 percent by volume, anesthetic grade) for sixteen hours to inactivate bacteria, yeasts, and fungi. Enteroviruses are ether resistant when treated under these conditions. Ether treatment was selected in preference to antibiotic treatment or membrane filtration because the former gave irregular inactivation of microorganisms, while the latter caused significant losses of viruses in the filters. Antibiotics were added to the cell sheets used during the assays to provide yet another protection against loss of a culture because of bacterial contamination.

When assaying for enteroviruses, samples were divided between two types of all cultures--Buffalo Green Monkey (BGM) kidney cells and African Green Primary Monkey Kidney (PMK) cells--in order to increase the chances for virus recovery. Hela cells (human cervical carcinoma cells) are required for assay of adenoviruses because the adenoviruses do not respond to nonhuman cells. Hela cells are preferable on the basis of price and availability but are somewhat less sensitive than human embryonic kidney (HEK) cells which could be used.

Samples were inoculated onto the cell sheets contained in one ounce bottles. The inoculum volume was from 0.1 to 0.2 ml per bottle. The bottles were then gently rocked back and forth for approximately one hour to allow the viruses to adhere to the cell sheets. Then, the cells are covered with an agar overlay and incubated for the required time.

An entire sample was always tested, but only one-half was tested at a time. The other half was kept frozen for two purposes: 1) to serve as a safeguard against loss of an entire sample in the event of problems (toxicity, contamination, etc.) encountered during the tests and 2) to provide confirmation of virus recovery if any were found during the assay of the first half of a sample.


Coded, Frozen Sample Received
Storage at -80°C Until Analyses Started
Sample Isotonic, Bacteriologically Sterile
One-Half Sample
Buffalo Green Monkey Culture
One-Half Sample
African Green Primary Monkey Kidney Culture
*Plaque-Development Procedures Involved Cell Monolayers Incubated at 37°C for 14 to 24 days.
Plaques Formed
Plaque(s) "Picked" and Passed to Fresh Monolayer (Same Type as for Original Plaque)
Cytopathic Effect Obtained, Virus Isolate Confirmed
Virus Isolate Identity Determined-Intersecting Pool, Serum Neutralization Tests Using Lim, Benyesh-Melnick (LBM) Pools
No Plaques Formed
Results-Negative

*Controls for non-virus contaminants, adventitious virus, monolayer integrity-monolayers inoculated with sterile diluent and overlaid with overlay media.

Figure 4. Test procedures used for virus analysis of Occoquan samples.

Plaques were cloned and propagated on additional cell sheets. The cells were lysed and centrifuged, first at low speed to remove debris and then at high speed to remove the viruses. The supernatant liquid was removed, and the viruses were washed and recentrifuged. The purified viruses were then subjected to the antisera-pools (Lim, Baenish-Melnick) for typing.

Conventional serum neutralization tests (10) were used for typing. The isolate was passed to large-area tissue cultures under liquid overlay. After incubation at 37.5°C for up to several days after which extensive CPE's were evident, the cells were lysed by repeated cycles of freezing and thawing. The liquid, containing progeny virus from the lysed cells, was freed of extraneous cell debris and other solids by low-speed centrifugation.

The product was titered over a range of serial dilutions to determine its virus concentration. An aliquot (0.025 ml) of the appropriate dilution was reacted with an excess of the appropriate antiserum pools and transferred to tissue cultures in microtiter plates. These were incubated and observed for CPE's. Observed patterns of neutralization, positive and negative controls were recorded and compared to establish the virus identity (10).

Reporting of data was on the basis of "CPE-positive" or "CPE-negative" and by type or, more commonly, by the number of PFU's per unit volume for each specific type of virus recovered. The minimum time required for assay and typing was approximately one to two months.

RCT Marker Test Procedure--

Virulent strains of poliovirus type 1, such as the Mahoney strain, replicate as well, or nearly as well, at 40°C as they do at 37.5°C. Avirulent strains such as LSc, replicate poorly, if at all, at 40°C. To establish the "T-Marker" characteristic or, more appropriately, the "reproductive capacity temperature" (RCT) characteristic, of a polio 1 virus, a single preparation is plated at several dilutions onto a common lot of tissue cultures. A portion of these is exposed to 40°C temperature before incubation at 37.5°C. The remainder is incubated at 37.5°C without the 40°C exposure. The rate of replication, shown as the number of PFU's, is measured for each. A calculation is made (See Table 8, Results Section) using Mahoney as the standard virulent type 1 poliovirus (R=100) and LSc as the avirulent standard (R=0). Calculated R values from 0 to 30 are considered typical of avirulent strains, whereas R values from 60 to 100 are considered typical of virulent strains. Other values are simply regarded as intermediate.

EPA Virus Group's Procedures

Concentration Equipment and Field Techniques--

The equipment used by EPA's Virus Group is basically that shown in Standard Methods, 14th edition (7), page 971. The specific details regarding their equipment and basic operating procedures used for concentrating viruses from large volumes have been discussed in detail by Hill et al. (6). A summary of their procedures follows.

Sterilization of equipment--The virus concentrator (proportioner unit, mixing chamber, hoses, and acid and sodium thiosulfate additive containers) was decontaminated with acidic calcium hypochlorite. The additive containers were filled with approximately 500 mg/l chlorine solution to which HCl has been added to reduce the pH to ≤ 6. This solution was dosed into tap water through the virus-concentrator proportioner and through a second proportioner placed between the tap water faucet and the inlet hose of the virus concentrator. This arrangement exposed the entire system to a decontamination solution of 5-10 mg/l hypochlorous acid, the most virucidal form of chlorine. The hyperchlorinated tap water is pumped through the system for 10 to 15 minutes, and then the ends of the hoses are covered with foil to maintain the disinfected state.

Adjustment of the tap water pH--Immediately prior to a sampling run, the volume of 0.2 Normal (N) HCl needed to reduce 220 ml of the water to be sampled to pH 3.5 is determined. For a standard 500 gallon water sample, a volume of 12 N HCl equal to 100 times the volume determined above is added to 22 liters of tap water in the acid additive container. When this solution is proportioned into the water sample at a 1:100 dilution, it achieves a pH of 3.5.

Neutralization of chlorine in water--A filter sterilized stock solution of sodium thiosulfate (STS) (75.6 g/100 ml) is prepared. One hundred and fifty ml of this stock solution is added to 22 liters of the water to be sampled in the second additive container. This mixture is dosed into the water to be sampled at a 1:100 dilution with two additive pumps so that STS is introduced with each stroke of the proportioner. The STS added is sufficient to neutralize approximately 7 mg/l chlorine.

Sampling procedure--The faucet from which the water sample will be drawn is flamed and the decontaminated hose is attached. Water is passed through the system to flush the residual chlorine remaining from the decontamination procedure. Also the effluent water is checked for sufficient pH adjustment (3.5 to 4.0) and chlorine neutralization. When accomplished, the heat-sterilized, virus-adsorbing filter unit (7-inch Balston filter with 8 μm porosity in a glass and stainless steel housing) is inserted aseptically into the system via quick disconnects. The flow is adjusted to 3-5 gallons per minute (gpm) and the sampling is begun. The effluent water is checked periodically for pH and chlorine neutralization. Corrections are made if needed. Water is also collected before and after the run for turbidity determinations.

Elution of virus adsorbing filter--At the end of the sampling period, the filter unit is aseptically removed and connected to a sterile pressure vessel which contains one liter of sterile glycine buffer at pH 11.0 to 11.5. The glycine eluent is pushed through the adsorbing filter and a smaller porosity clarifying filter with nitrogen gas. The eluate is immediately lowered in pH to 8.0 to 9.0 with approximately 300 ml of glycine buffer pH 1.1. The eluate is collected in a sterile, screw-cap, polypropylene bottle. The cap is sealed with tape and the container is placed on wet ice until it is returned to the laboratory.

Virus Assays--

Reconcentration of eluate--If the eluate is to be reconcentrated within 12 to 16 hours after arrival at the laboratory, it is stored at 4°C; if not, it is stored at -70°C. Just prior to processing, the pH meter is standardized with pH 4.0 standard buffer and the electrode subsequently sterilized with 10 mg/l Cl_2 solution for 30 seconds and neutralized with sterile sodium thiosulfate solution. Processing begins by transferring sample to a 1,500-ml beaker with stir bar in a laminar flow hood. The stirrer is started and glycine buffer at pH 1.1 is added slowly until pH 3.5 is reached. A 0.05 M solution of $AlCl_3$ is added to achieve 0.005 M $AlCl_3$.

The sample is then filtered by suction through a "sandwich" of 5 µm and 1 µm Cox Filters. After all the sample has passed, 25 ml of sterile, physiological saline (pH 3.5) is passed to wash filters.

The filter assembly is transferred to a 125-ml, side-arm flask and 14 ml of glycine buffer at pH 11.1 is passed under suction. The eluate is caught in the side-arm flask which contains 2.6 ml of pH 1.1 glycine buffer, 2.0 ml of 10X nutrient broth and 1.4 ml of 10X Hank's basic salt solution. At no time in the elution or reconcentration step was the sample allowed to remain at pH 11.0 to 11.5 for more than five minutes (usually less than three minutes). The total volume of approximately 20 ml is divided equally and placed in plastic centrifuge tubes and immediately frozen at -70°C. The entire process is usually accomplished within 20-30 minutes. This can vary depending upon the ease at which the sample passes the filters. All operations are carried out in laminar flow hoods.

Isolation and identification of viruses from the sample--The 20 ml, reconcentrated sample is frozen at -70°C until appropriate cell culture is available. Generally, the sample is divided equally and the entire sample is placed on monolayers of three cell types: Barron's line of African green monkey kidney cells at passage number approximately 130 (BGM), McAllister's line of human rhabdomyosarcoma cells (RD) and primary African green monkey cells obtained from Flow laboratories. The cell monolayers are observed for 14 days for CPE. If no CPE is visible by the 14th day, the cells and media are frozen and thawed and five ml of this solution is passed to each of three new monolayers. If no CPE develops in 14 days of the second passage, the sample is considered negative for virus. If CPE is observed, the cell bottle(s) is removed from the isolation lab and identification procedures begun.

Personnel Surveillance

During Occoquan-II, the Carborundum field personnel, and anyone else entering the mobile laboratory used for the virus concentration work, were examined periodically to determine if they were shedding viruses. Swabs, both rectal and throat, were taken with a sterile, cotton-tipped swab, placed in a sterile, screw-capped test tube containing 1.0 ml of sterile nutrient broth and returned to UNH (Spaulding Life Sciences Laboratory) for assay. The swab tests were performed at a frequency which was practical (from the standpoints of both cost and time-and-effort) for the purpose of assisting in the interpretation of data in the event a concentrated sample was positive for virus.

During Occoquan-II, rectal swabs were collected monthly and sera specimens were collected quarterly on each EPA participant in the study. The swabs were stored at -70°C in 1.0 ml of sterile nutrient broth containing 3X antibiotics. During the comparative seeded studies, rectal swab collection was altered to coincide with the initiation of an experimental run. These stored specimens were assayed for virus or specific antibody only when such data were needed to determine the occurrence of sample contamination, i. e. when field samples yielded virus isolations or aberrations occurred in the seeded study results. Specimens were assayed in BGM and RD cells. The inoculated cultures were observed for 14 days for CPE and blind passaged to fresh cells and observed for an additional 14 days before declaring them negative for virus.

COMPARATIVE STUDIES: FIELD SAMPLING AND ANALYSIS

During Occoquan-II, EPA's virus group participated on thirteen occasions in a collaborative, field-sampling effort with The Carborundum Company's virus group. The first studies were in July, 1978, when eleven samples were collected by each group from the raw water and finished water at FCWA's New Lorton treatment plant and at two sites in the distribution system. In October, 1976, samples were taken from a local sewage treatment plant. Two sites were selected: 1) the effluent from the plant's secondary clarifier prior to its entrance into a storage lagoon (detention time approximately thirty days) and 2) the chlorinated effluent from the lagoon itself.

Each field team processed approximately 100 gallons of treated water from FCWA's system through their concentrators and completed the necessary elution steps in the field. When the sewage treatment plant studies were conducted, only a relatively small volume of the extremely turbid effluent could be forced through the concentrators (2.2 gallons and 8.5 gallons for Carborundum and EPA, respectively), but more of the lagoon effluent (50 and 100 gallons, respectively) could be concentrated.

After the elution and reconcentration steps were completed, each party sent a portion of each of their concentrates to the other for viral assay. The Carborundum Company's concentrates from the sewage treatment plant study included one from the clarifying filter and one from the adsorbing filter. All procedures, including those for storage and shipping, were as previously described.

Prior to the initial comparative-sampling and analysis study, the BGM cell line used at UNH was shipped to the EPA laboratories in Cincinnati. The purpose was to eliminate one possible source of variability between the two laboratories, namely the degree of susceptibility of different cell cultures to any viruses which might be recovered.

ADDITIONAL FIELD STUDIES

Two additional short-term studies were conducted in an attempt to recover natural viruses from a public drinking-water supply. While these studies were conducted only by the VPI/Carborundum group, portions of the final concentrates were sent to EPA for virus analysis as needed. It is known that alum flocculation will concentrate a majority of viruses present in water and, because flocculation is a principal process in the treatment of water for public consumption, it was believed virus recovery might be possible from back-wash water collected from a water treatment plant filter and from the unwashed filter media itself. It seemed reasonable to assume that virus recovery from the water would be enhanced by the concentration potential the alum floc and the filter media provided.

Studies Associated With a Water Treatment Plant Filter

A pipe-and-valve assembly was placed in the waste line from the filter gallery that carried backwash water (including the surface wash water) to waste and during backwashing of a filter, the water to be concentrated was pumped into a tank. Because of difficulties getting water out of the valve during backwash (Bernoulli's principle), it was necessary to pump from the line to a large tank, and consequently, only about 50 gallons could be collected before the backwash cycle was completed. During the concentration step, the water in the tank was mixed continuously with compressed air to keep floc suspended.

The tank used to collect the water prior to carrying out the concentration step of the virus-recovery procedure was a 500-gallon, polyethylene tank. It was initially sterilized with a high concentration (50-75 mg/l) of chlorine at an approximately neutral pH. Thereafter, at the end of an experiment, the tank was thoroughly cleaned with tap water having a residual of 3-4 mg/l and then dechlorinated. The rigorous sterilization procedure was not regarded as necessary between experiments.

In addition to the backwash water, samples were taken also immediately before and after a backwash cycle from pipes below the filters and pumped directly into the Aquella^{tm} concentrator. Two filter underdrains collected water passing through the filter, one for each half of the filter, and. consequently, the pre- and post-backwash samples were water from only one-half of a given filter. When a filter was backwashed, the wasteline carried washwater from the entire filter.

Study of Filter Media

A method was developed by The Carborundum Company to examine the anthracite from one of FCWA's mixed-media water filters. Several attempts were made to recover poliovirus (LSc-1) from seeded samples of anthracite samples collected from an unwashed filter. The method which was adopted allowed 67 percent recovery of the seeded virus.

A weighed sample (six to eight pounds) of the anthracite filter medium was placed in a 4-liter beaker and eluted twice by covering it with pH 10.5, 0.5 M glycine. The decanted solution was reconcentrated by floc formation with addition of 0.003 M $AlCl_3$, followed by neutralization with Na_2CO_3 and centrifugation. The pellet was eluted with pH 10.5 glycine, and neutralized, then fetal calf serum was added. The final concentrate was divided equally for analysis by both EPA and The Carborundum Company virus groups. The experiment was conducted twice.

SAMPLING FREQUENCIES

Occoquan-I

Seeded Samples--

During Occoquan-I, a total of twelve field experiments, two at each sampling site, were conducted to determine the effectiveness of methods used by The Carborundum Company for concentrating and assaying for viruses. In these tests, large numbers of viruses (10^5-10^6 PFU, polio type 1, adenovirus, and reovirus) were added to the thiosulfate reservoir (one virus type at a time) and were injected into the stream of water taken from the sampling site as it was being forced through the adsorbing filter. In most instances, from 85 to 100 gallons of water were used at the finished-water sites. Much less (50-85 gallons) of the stream samples could be forced through the filters because of the high suspended solids in the water.

Natural Samples--

Table 4 shows the number of water samples collected for virus analysis during a ten week period from June 2 through August 27, 1975. The sampling was restricted to this period during Occoquan-I because it is this time of year that viruses are shed by humans in large numbers. Because treated sewage enters the reservoir (via Bull Run), the likelihood of virus recoveries was regarded to be greatest during the summer months.

After the concentration step was completed, the preparation of the final concentrate for shipment to UNH was by the routinely used procedures which have already been described.

Occoquan-II

Environmental Samples--

During Occoquan-II, the environmental sampling for viruses was restricted to the enteroviruses (polio, Echo, and Coxsackie). (Reovirus and adenoviruses were included in Occoquan-I.) Table 5 shows the numbers and frequencies of the samplings from June through October, 1976, and in March, 1977. During July, 1976, the EPA virus group participated in a joint sampling and analysis program at the sites at the treatment plant and in the distribution system. They returned in October, 1976 to jointly sample at the sewage treatment plant.

TABLE 4. NUMBERS OF SAMPLES ASSAYED FOR [a]ENTEROVIRUSES, REOVIRUS, AND ADENOVIRUS DURING OCCOQUAN-I, JUNE - AUGUST, 1975

Sampling Site	Number of Samplings for Virus Type Shown											
	June			July			August			June-August		
	Entero	Reo	Adeno	Entero	Reo	Adeno	Entero	Reo	Adeno	Entero	Reo	Adeno
A Bull Run Above [b]STP Discharges	5	4	1	8	4	2	4	0	0	17	8	3
B Bull Run Below [b]STP Discharges	8	8	2	5	2	1	4	0	0	17	10	3
C Raw Water, RCWA	4	4	2	3	2	1	3	0	0	10	6	3
D Finished Water, FCWA	4	4	2	4	1	1	2	0	0	10	5	3
E Distribution System	5	5	2	1	1	1	0	0	0	6	6	3
F Distribution System	5	5	2	1	1	1	0	0	0	6	6	3

[a] Enteroviruses include polio, Coxsackie and ECHO viruses

[b] STP = Sewage Treatment Plant

TABLE 5. NUMBERS OF ENVIRONMENTAL SAMPLES ASSAYED FOR [a]ENTEROVIRUSES DURING OCCOQUAN-II, JUNE, 1976 - MARCH, 1977 BY THE CARBORUNDUM COMPANY

Sampling Site	Number of Samplings for the Month Shown						
	June	July	August	September	October	March	Total
[b]A Bull Run Above [c]STP Discharged	0	0	0	0	1	0	1
[b]B Bull Run Below [c]STP Discharges	0	0	0	0	1	0	1
[b]C Raw Water, FCWA	3	5 [d](3)	6	0	1	2	17
D Finished Water, FCWA	3	5 [d](3)	4	3	0	3	18
E Distribution System	2	6 [d](2)	5	2	0	3	18
F Distribution System	2	5 [d](2)	6	2	2	0	17
[b]H Open Reservoir Site F	0	0	0	0	0	2 [d](2)	2
North Side STP	0	0	0	0	2	0	2

[a] Enteroviruses: polio, ECHO, Coxsackie

[b] Both the clarifying and adsorbing filters were eluted and the eluates reconcentrated and assayed

[c] Sewage Treatment Plant. [d] Numbers indicate times EPA participated in joint-sampling and analysis of samples.

Additional Field Studies--

On June 2, 3, 6, 7, and 10, 1977, samples were collected from the filter backwash waste line and from clean water passing a filter at FCWA's New Lorton facility. The anthracite sample was taken for analysis during this period also. The EPA did not participate in the sampling program during this period but did analyze the concentrates of these samples provided by The Carborundum Company.

Seeded Samples--

Both The Carborundum Company and EPA jointly participated in studies designed to evaluate the sampling, handling, and assay procedures used by each group. These studies were conducted in the Number 2 Pump House at the New Lorton facility from December 3 through December 21, 1976, and from February 15 through March 1, 1977. A total 35 samples was collected by each group. A detailed description of this program is presented in the following section.

SUPPLEMENTAL WATER-QUALITY DATA

Supplemental water quality data required by the contract for both years of the study included chlorine residuals (free and total), pH, temperature, bacterial counts (coliforms and total plate counts, the latter on finished water only), and notes concerning any unusual characteristics such as high turbidity and any unusual color or odor. All methods were according to Standard Methods for the Examination of Water and Wastewater (7). During the second year, monitoring of finished waters for total organic carbon (TOC) was required, primarily for use in interpreting trihalomethane data presented in Part I of this report.

Chlorine Residuals

Both total and free residuals were determined by the DPD Ferrous Titrimetric Method Sec. 409 E (7). The reliability to 0.1 mg/l was verified by testing aliquots of samples by the Amperometric Titration Method, Sec. 409 C (7). The titrimetric method was suitable for analysis of all but the most turbid samples, as are common to the tributary streams of the reservoir after a rainstorm, but the residuals there were less than 0.1 mg/l on all occasions. The principal sites where chlorine residuals were important were those where finished water samples were collected.

pH Value

The pH of all samples was determined according to Standard Methods, Section 424 (7). A portable pH meter was used and standardized before each use with commercially available buffer solutions.

Temperature

Temperature was measured according to Standard Methods, Section 212 (7) with a mercury-filled Celsius thermometer calibrated in increments of 0.1°C. The thermometer was placed in the sample immediately after collection and allowed to equilibrate before the reading was taken.

Bacterial Counts

Coliforms--

The Multiple-Tube Fermentation Technique, Section 908, was used for both total coliforms (908 A) and fecal coliforms (908 C) (7). Both the presumptive and confirmed tests were performed using five transplants of aliquots from the serial dilutions. Occasionally, the completed test was performed to verify the identification reliability. Both raw and finished-water samples were analyzed for coliforms, and the dilutions and media inoculations were performed in the field. The tubes were returned to the laboratory for incubation within four to six hours.

Total Plate Counts--

The standard plate count procedure (Section 907) (7) was performed only on finished waters. Plates were inoculated in the field at the time samples were collected and returned to the laboratory for incubation within four to six hours.

Total Organic Carbon (TOC)--

The TOC concentrations were determined (during Year 2) by analysis with a Beckman Instruments 915A TOC Analyzer. Potassium phthalate standards were used so that full-scale deflection on the strip chart recorder was 20 mg/l, and the reproducibility of analyses of standards was within 0.2 mg/l of the true concentration. During the latter part of the study period, the laboratory acquired a Dohrmann/Envirotech Model 54 TOC Analyzer, permitting analyses with precision to less than 50 μg/l TOC.

COMPARATIVE STUDY WITH CODED, SEED-VIRUS SAMPLES

Overview

Because viruses were reported to have been recovered from finished water during Occoquan-I (to be discussed in this report) a carefully controlled, large-scale experiment was devised to evaluate the virus recovery methods and control procedures involved in this study. The study was designed so that procedures used by the virus monitoring and assay teams from both the EPA and The Carborundum Company could be evaluated in an objective manner. A third group from the California State Department of Health's Viral and Rickettsial Disease Laboratory (CSDH) prepared and coded the viral concentrates and "blanks" (virus-free samples) to be used in the studies.

A system was designed which would provide large quantities of virus-free water which could be held in a large, sterile reservoir. On twenty-five occasions, a vial containing either a blank or a virus concentrate (virus type and titer unknown to experiment participants) was opened, diluted, and divided equally between the two parties for mixing in the thiosulfate reservoir associated with their concentrators. On ten occasions, the contents of a coded vial was diluted directly in the tank containing the heat-treated and cooled water and thoroughly mixed with a large agitator (Lightnin' Corporation). Each virus group had the responsibility for passing approximately 100 gallons of the test water through their concentrator and for preparing their respective concentrates by their particular procedures. Each group's assay laboratory was to receive one of these portions for virus assay.

The coding system devised assured that neither field party knew the contents of any vial sent from CSDH, nor did their respective laboratory personnel know which group had prepared the concentrates that they were analyzing. After all data had been reported to the EPA Project Officer in coded form, he and another party decoded it for analysis.

Sample-Handling Procedures

Figures 5 and 6 show schematically the flow of sample vials, field concentrates, and data. Specific details are as follows:

1. Vials, containing either virus concentrates or blanks, were prepared and coded by CSHD and sent frozen to the OWML director (B, Fig. 5). They were stored at -70°C in a Revco freezer (Model ULT-7100B, 7 cubic ft) at FCWA's New Lorton treatment Plant. Before the actual samples were sent, a shipment of blanks was made to verify that the established procedures would be successful, i.e., that samples would be received unbroken and frozen without delay. Samples (packed in dry ice) arrived by air and were taken by truck to the Federal Express Office in Alexandria, Virginia, where they were picked up by the OWML director.

2. Samples were recoded (without thawing) by the EPA Project Officer, the FCWA laboratory director, and the OWML director (step 2, Fig. 5).

3. On the day of an experiment, the contents of one vial were thawed and diluted into the thiosulfate reservoirs or added directly to the heat-sterilized water reservoir by OWML personnel (Step 3, Fig. 6). During the thiosulfate experiments, each party added one-half of the diluted vial contents to their reservoirs and measured the volume remaining at the end of the run in order to determine the input virus titer.

4. The field concentrates were then sent to the field teams' respective assay laboratories for further reconcentration and separation into two aliquots which were then returned to FCWA and stored in the Revco freezer.

5. The EPA Project Officer and the FCWA laboratory director recoded the vials once again (Step 4, Fig. 6) and shipped back to each assay laboratory one-half of a concentrate prepared both by its own personnel and by those from the other laboratory (Step 5, Fig. 6).

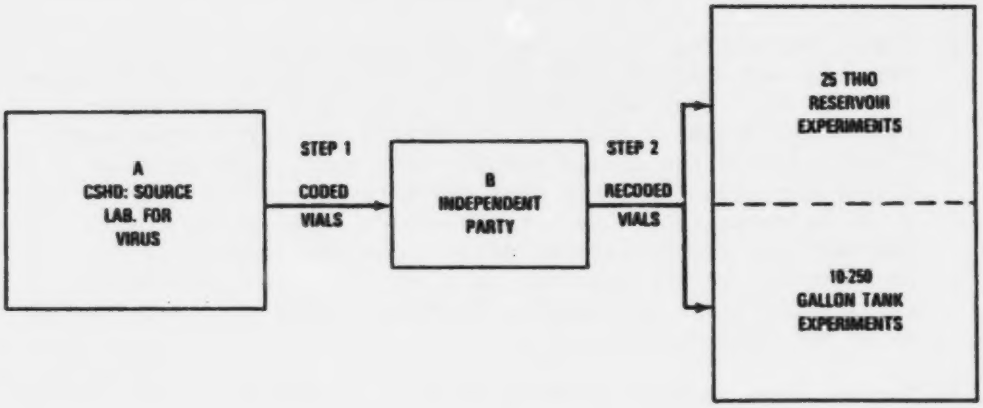

A
CSHD: SOURCE
LAB. FOR
VIRUS
STEP 1
CODED
VIALS
B
INDEPENDENT
PARTY
STEP 2
RECODED
VIALS
25 THIO
RESERVOIR
EXPERIMENTS
10-250
GALLON TANK
EXPERIMENTS

Figure 5. Schematic for Providing Virus Input Concentrates for Each Experiment.

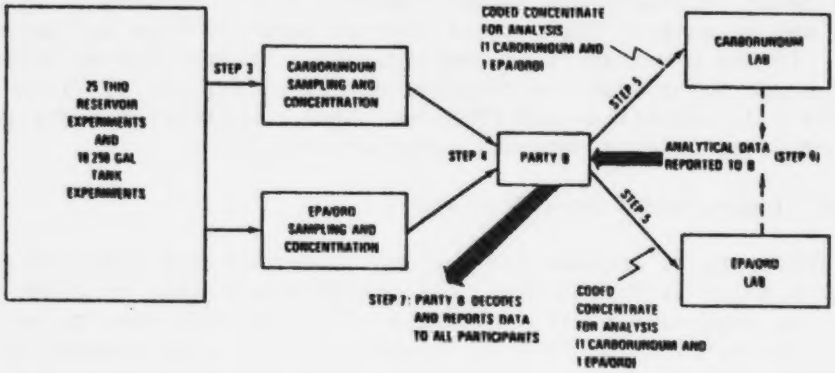

25 THIO
RESERVOIR
EXPERIMENTS
AND
18 250 GAL
TANK
EXPERIMENTS
STEP 3
CARBORUNDUM
SAMPLING AND
CONCENTRATION
EPA/ORD
SAMPLING AND
CONCENTRATION
STEP 4
PARTY B
CODED CONCENTRATE
FOR ANALYSIS
(1 CARBORUNDUM AND
1 EPA/ORD)
STEP 5
CARBORUNDUM
LAB
ANALYTICAL DATA
REPORTED TO B
(STEP 6)
STEP 5
EPA/ORD
LAB
STEP 7: PARTY B DECODES
AND REPORTS DATA
TO ALL PARTICIPANTS
CODED
CONCENTRATE
FOR ANALYSIS
(1 CARBORUNDUM AND
1 EPA/ORD)

Figure 6. Schematic for Processing: Steps in the Comparative Sampling, Concentration, Analysis, and Reporting of Data.

6. The final results (PFU's per ml, total volume of concentrate, and, on occasions, virus type when an isolate was recovered) were then sent to the EPA Project Officer. He then decoded the data and sent copies to each of the principals in the project for analysis (step 7, Fig. 6).

Role of California State Health Department

CSHD was selected as the independent party in this project, both because their personnel are internationally known, extremely capable virologists and because of their total lack of personal bias in the project itself. It was necessary that some competent independent party be found that would agree to provide the required number of ampules of viruses--correctly titered and identified--and CSHD met these requirements. The work by CSHD was performed under a separate contract with EPA.

Number, Types, and Titers Required--

The contract between EPA and CSHD required that vials of two poliovirus types at three different titers be prepared and coded by CSHD. In addition, replicate vials, identical to those which were to be shipped, were to be retained by CSHD as a backup in the event samples were lost in shipment or otherwise rendered unusable. Table 6 shows details of the numbers and contents of vials to be sent and the intended purpose of each. The listed titers were close to the target titers called for by the contract, and indicated valid performance by CSHD.

The samples intended for direct analysis by both assay laboratories were provided so that a comparison could be made at the end of the study to the original titers as determined by CSHD.

Specifications for Preparation of Virus Materials--

The following instructions for preparing the virus materials were given in the contract between EPA and CSHD:

1. The viruses shall be grown on any appropriate cell system.

2. The viruses shall be titered on the BGM cell system furnished from Dr. Metcalf's laboratory.

3. The stock virus should be harvested at the point of maximum virus yield and rapidly frozen and thawed two times to release the virus. This crude virus preparation should be refined and rendered monodispersed by the following steps (modified after Ver *et al.*, *J. of Vir. 2*: 21, 1968):

   a. Preparation of Membrane Coating Solution (MCS)-Prepare a 10 percent solution of fetal calf serum in distilled water. Filter, under lab pressure, this solution through a series of the following filters: An AP20 prefilter, a 0.22 μm and a 0.05 μm Millipore (MF) membrane. Make the filtrate isotonic with 10X Earls balanced salt solution.

TABLE 6. CONTENTS OF [a]VIALS PREPARED BY CALIFORNIA STATE HEALTH DEPARTMENT LABORATORY FOR USE IN COMPARATIVE SAMPLING STUDY

[b]Vial Contents	Target Virus Titer PFU	Number of Vials for Use as Shown[c]			
		Dilution in Thio Reservoir	Dilution in Sterile Reservoir	EPA, Direct Analysis	Carborundum, Direct Analysis
Polio 1	53	5	3	5	5
Polio 1	300	9	4	5	5
Polio 2	250	1	1	5	5
Polio 2	2.9×10^6	1	1	5	5
[d]Blank	-	9	1	5	5
		$\Sigma = 25$	$\Sigma = 10$	$\Sigma = 25$	$\Sigma = 25$

---

[a] Total Volume, 5 ml.

[b] Viruses in sterile, nutrient broth (Difco #003-01), prepared with Sterile Hanks BBS, and buffered to pH 8.0

[c] Does not include the vials prepared and retained by CSHD as reserve back-up.

[d] Nutrient broth autoclave-sterilized at 121°C for 15 minutes

b. Pretreatment of Filters with MCS - Using pressure filtration, slowly pass 10 ml of MCS through two sets of a 47 mm, 0.22 μm, and a 0.05 μm MF membrane in series. Rinse the membranes with 5 ml of Tris buffer. Discard the filtrate and use these two sets of membranes to filter the virus stock suspension. (Prepare these filters shortly before use).

c. Filtration of Virus Suspension - Centrifuge the stock virus suspension at low speed (∿ 2000 RPM) for 15 minutes. The supernate should then be removed and adjusted to pH 8.0 with a nonbicarbonate buffer, e.g., Tris. The supernate should then be pressure filtered in the cold through one series, then the other series of the 0.22 μm/ 0.05 μm treated membranes. (Filtration may take several hours). Store the filtrate in small volumes in sealed ampules at -70°C until ready for use.

4. The virus dilution and suspension fluid shall be sterile nutrient broth (Difco #003-01) prepared with sterile Hanks BBS, buffered to pH 8.0, rather than distilled water.

5. The desired virus material vials shall be produced and titered in following fashion:

a. The filtrate, produced according to step 3.C., will be assayed by 10 replicate titrations. For example, this may be accomplished by diluting the filtrate to give a range of 10-30 PFU/ml then by inoculating ten 25-cm^2 cell monolayers with one ml each of the diluting filtrate. Based on this titration appropriate pools can be prepared to give the desired titers. Five ml each of the pools should be placed in 10-ml ampules and flame sealed. They should be checked for leakage by an appropriate procedure and should be frozen at -70°C.

b. For the desired 2.5 X 10^6 PFU titer, it is recognized that this may prove impracticable to attain and therefore the highest attainable will be acceptable.

c. To obtain official titers, 10 ampules at each virus concentration level will be removed from the freezer at random.

(1) For the 2.5 X 10^6 PFU titer, the vial contents may be diluted to give 10-30 PFU/ml and a 1/ml sample taken for assay.

(2) For the 250 PFU and lower levels, the entire vial contents should be assayed.

(3) As a guide the contractor should try to have the mean plaque count come within ± 2 times the square root of the desired number.

Preparation of Pasteurized Dilution Water

A rather elaborate system was designed for use during the comparative study to ensure that the test water was free of naturally occurring viruses. This detail was of utmost importance because the contamination question is always a concern in virus monitoring and assay research. In the following paragraphs, the design and operation of the system will be discussed.

The Equipment--

A diagram of the units comprising the water-pasteurization system is shown in Figure 7. The specifications for these units were as follows:

1. Packaged water chiller - Kold Wave Model LC500A, 5-ton unit. Included freon compressor, shell-and-tube heat exchanger, electrical box with motor starter and thermostat. The unit could be installed either outdoors or inside.

2. Plate heat-exchanger - Chester Jensen Company, Model HTW with type 316 stainless-steel plates. Unit could be mounted either on the wall or the floor. Heating and cooling surface area 15 square feet. Equal flow on each side of the plates (hot and chilled water) was provided to accomplish 80-83 percent regeneration. The head loss through the unit at five gallons per minute was 30 pounds per square inch.

3. Water heater - Hi-Power Model 120-18, Commercial electric type, 120 gallon capacity, manufactured by Lochnivar. Included thermostat, heavy duty tank, high-temperature cutoff, and insulation.

4. Storage reservoir - The pasteurized water was stored in a 250-gallon, polyethylene tank (United Utensils Co., Model 260-S, Port Washington, N. Y.) equipped with a loose lid and 3/4-inch, IPS, female, threaded connection. Two take-off spigots were installed, one for each of the two virus concentrators. The influent line terminated about one foot above the surface of the filled reservoir, providing an air break which ensured that there was no opportunity for back flow.

5. Mixer - A high-speed mixer (Model ND-1A Lightnin' Agitator, 1/4-horsepower, carbon-steel shaft and 2-prop shaft) was mounted on the tank to provide the mixing required for those studies (ten in all) when the virus concentrate was added directly to the reservoir prior to passage of the water through the virus concentrators.

Sterilization of Storage Reservoir--

Procedures for sterilizing the 250-gallon tank before each of the seeded experiments where virus were added to the tank itself were as follows:

a) Fill tank, add one quart commercial sodium hypochlorite bleach, adjust pH to less than 7.0 with HCl. Let stand overnight (calculated dose, 220 mg/l chlorine).

b) Add sodium thiosulfate to dechlorinate.

c) Drain tank and rinse with water from the pasteurization system.

d) Ensure chlorine residual is zero.

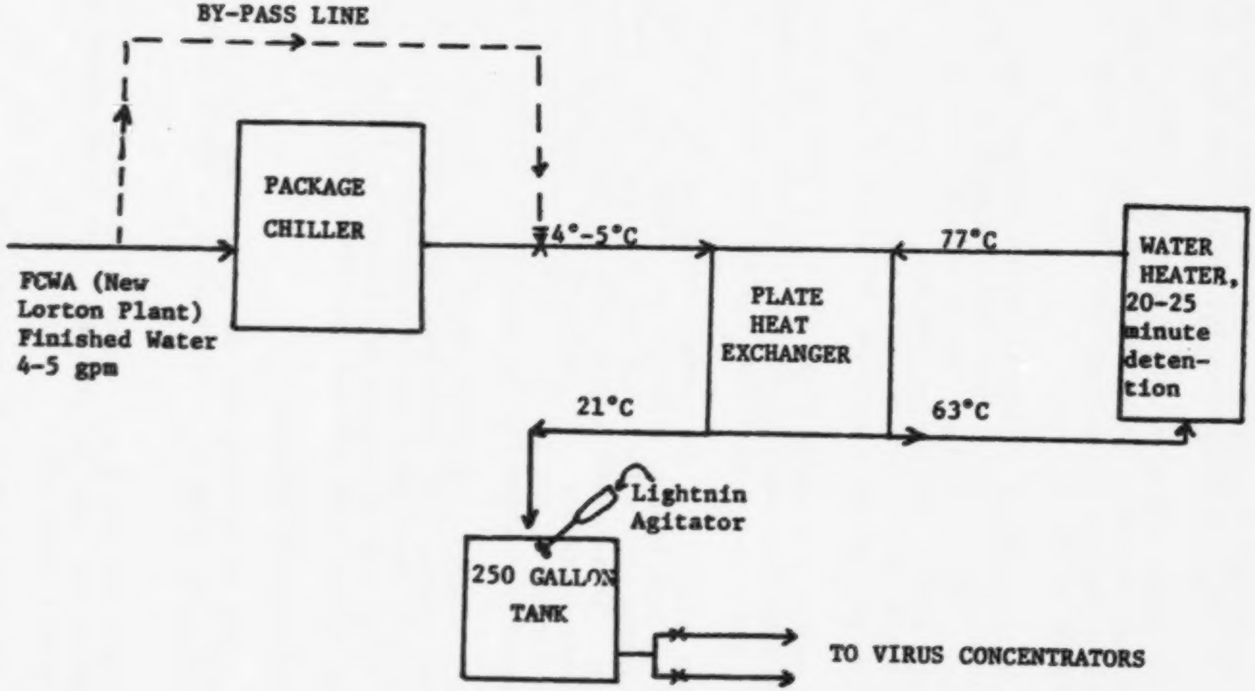

BY-PASS LINE
PACKAGE CHILLER
FCWA (New Lorton Plant) Finished Water 4-5 gpm
4°-5°C
PLATE HEAT EXCHANGER
77°C
WATER HEATER, 20-25 minute deten-tion
21°C
63°C
Lightnin Agitator
250 GALLON TANK
TO VIRUS CONCENTRATORS

Figure 7. Diagram of the Water-Pasteurization System and Storage Reservoir. All temperatures are design values.

e) Fill tank with pasteurized water.

After each experiment when viruses were added to the thiosulfate (the first 25 experiments), the superchlorination of the tank was not required, so only a pasteurized-water rinse was used to prepare the tank for the next day's experiment.

Mixing Study--

To ensure that viruses added directly to the storage reservoir prior to the concentration step were completely mixed, a dye study was performed. A stock solution of methylene blue was prepared and introduced into the tank with the Lightnin' Agitator operating. The dye was dispersed rapidly and no dead spaces could be seen. Complete mixing appeared to be accomplished within two minutes, but when seed viruses were added directly to the tank, the mixing time was exactly five minutes.

Pasteurization Procedure--

Finished water (under line pressure) was passed through an in-line flow-meter, at the rate of no more than four gallons per minute (gpm) through the chiller (to cool it to 4-5°C if necessary) to one side of the heat exchanger. If the influent water temperature was less than 4°C, the chiller was bypassed. (The chilled water in the heat exchanger was used to cool the heat-pasteurized water to 21°C before it was stored in the 250-gallon tank.) In the heat exchanger, the water was warmed to approximately 60°C before it entered the water heater. At a low of 4 gpm, the theoretical detention time in the heater was 30 minutes. From the water heater, the water (approximately 77°C) flowed through the other side of the heat exchanger where it was cooled to approximately 21°C. On occasions when the effluent from the water heater was not as warm as desired, the flow rate was reduced below 4 gpm to provide greater detention. The thermometer which registered the temperature in the tank probably was several degrees higher than that recorded. The pasteurized water was collected in the storage tank. The time required to fill the tank in preparation for an experiment was from one to one and one-half hours, depending on the flow rate.

Description of a Typical Experiment

The following instructions were followed by all field personnel in the execution of the seeded-virus experiments.

Handling of Coded Vials Prior to Concentration Steps--

1. Rinse hands with water treated with HCl to reduce pH to less than 1.0. Wear a sterile, gauze mask.

2. Select a coded vial from the freezer. Record its code number, then permit it to thaw completely at room temperature. Rinse its outside well with water, pH < 1.0.

3. Break vial and withdraw contents with a sterile, 10-ml Luer-Lok syringe equipped with an 18-20 gage needle. Dispense the contents into a sterile, one-liter graduated cylinder containing pasteurized water. Fill the vial several times with pasteurized water, withdrawing each rinse with the syringe and dispensing to the graduated cylinder.

If the diluted material is to be added to the thiosulfate reservoirs of the two field teams, proceed to Step 4. If the material is to be added to the 250-gallon tank, proceed to Step 5.

4. a. Fill the graduated cylinder to the mark with pasteurized water from the water treatment system. Mix the contents well by pouring back and forth into a second, sterile, graduated cylinder. Keep the vessels that contain the diluted material covered with aluminum foil when they are unattended.

   b. Dispense exactly 500-ml of the diluted virus concentrate into each of two, sterile containers having a capacity greater than 5.0 ℓ. These containers will be the thiosulfate reservoirs. The total volume of the thiosulfate in the reservoir should be 4.75 ℓ ± 5 ml. Use pasteurized water when preparing the thiosulfate.

5. Add contents of the graduated cyclinder directly to the 250-gallon tank containing approximately 250-gallons of pasteurized water. Rinse graduated cylinder and empty rinse water into the tank. Mix contents for five minutes with Lightnin' Agitator.

Waste-Handling Procedures--

1. After each virus team has passed approximately 100 gallons through their concentrator units, the excess water in the thiosulfate reservoir or the water tank was chlorinated (approximately 100 mg/1) and allowed to stand for one hour.

2. The waste was pumped to a 55-gallon drum containing hypochlorite solution. When the drum was full it was discharged to the sewer and flushed with tap water.

Additional Precautions and Clean-Up Procedures--

1. A large catchment basin was placed under the tank tap to collect any material that should leak out. If there was leakage and the depth in the catchment exceeded one-half inch, the experiment was to be terminated.

2. The catchment basin and 250-gallon tank were rinsed with a strong hypochlorite solution at the end of each experiment.

3. The work area was sprayed with a hypochlorite solution.

4. Responsible personnel remained in the area during an experiment to pour hypochlorite onto spilled material in case of an accident.

5. All glassware to be used was soaked overnight in acidified water, pH < 1.

Data Collection and Analyses Performed--

Prior to the initiation of the concentration step, and again at the end, the temperature, chlorine concentration (free and total), and pH of the pasteurized water were measured. Bacterial tests (only at the beginning) included total and fecal coliforms and standard plate counts. After the concentration step was completed, the remaining volume in the thiosulfate reservoir was measured and recorded. This information was not needed if the coded material had been added directly to the 250-gallon tank.

Procedures Following Initial Concentration Step

The adsorbing filters were eluted on-site by the field personnel. The Carborundum group carried out the gel-reconcentration procedure before the samples were shipped to UNH for further reconcentration. At UNH the concentrates were divided into two equal portions, refrozen, and returned to FCWA where they were stored in the Revco freezer. Later, the samples were assigned new codes by the Project Officer and FCWA Lab Director, and one of the two portions of each concentrate were shipped to the two assay laboratories to be put on the cell lines.

The EPA group returned its concentrates to Cincinnati where they were reconcentrated and divided into two equal portions. The procedures from that point on were identical to those just described.

Once the assay laboratories had received their samples, they followed the protocols previously described. Due to a misunderstanding, the UNH laboratory group failed to identify all the plaques they observed during analysis of both halves of the first twenty-five concentrates (when coded concentrates were added to the thiosulfate reservoir). Instead, only one representative plaque was picked and subjected to the routinely used typing procedures. The UNH group typed all plaques during analysis of the final ten experiments (when coded concentrates were added directly to the reservoir) except in those tests where the titers were 10^6 PFU. In these, several plaques were picked at random for typing. EPA identified all PFU except for the 10^6 PFU experiment.

Copies of all the data, code sheets, correspondence, etc., were delivered to each participating individual or team, and the entire series was decoded and summarized.

At the end of the 35 experiments, the CSHD group, which had prepared and titered the original concentrates, retitered the various virus lots to determine if changes had occurred during storage. These data were forwarded to the EPA Project Officer for use in calculations of recoveries by both of the principal participants in the project.

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SECTION 5

RESULTS

OCCOQUAN-1

Environmental Monitoring

During the first year of the study viruses were reported in seven concentrates, four of which were from finished-water samplings. All the isolates were identified as Polio 1. During that year, there was a total of 22 finished-water samples (Sites D, E, and F) and 44 natural samples (Sites A, B, and C) collected from June 2 through August 27, 1975 through this project. A summary of the locations and dates, along with the corresponding bacterial counts and chlorine residuals, is given in Table 7. All the isolates were from adsorbing filters. None of the clarifying filters were eluted during the first year's study. In addition to the samples provided for by this project, biweekly samples of raw and finished water were sampled for viruses by the OWMP and FCWA as part of their routine monitoring programs.

Selected data collected in the field during sampling and in the laboratory during the assay procedure are presented in Appendix Table A-1. Appendix Table B-1 contains all the supplemental data required by the contract.

RCT-Marker Studies

Because all the isolates were Poliovirus 1, there was concern that they were contaminants from either a failure to properly disinfect the Aquella™ concentrator or to improper handling of the sample after its collection and during the assay. An additional characteristic of the isolates--the rct-marker--was determined for all but one isolate (from Site F, June 3) and compared with those of other viruses, including the routinely used laboratory strains and that used in the seeding experiments conducted before the first environmental sample was taken. The results of the marker studies are presented in Table 8. These data are discussed in Section 6.

Seeded Samples

Results of the twelve tests where viruses were added to the influent water are presented in Table 9. As can be seen, the recoveries of poliovirus were quite good, though the adenovirus recoveries were much lower. Too, reovirus was never recovered. The reason is not known, and the problem was an enigma because The Carborundum Company's field teams have *recovered reoviruses in other studies.*

TABLE 7. SUMMARY OF VIRUS RECOVERIES AND SUPPLEMENTAL WATER QUALITY DATA DURING JUNE THROUGH SEPTEMBER, 1975

Date	Sampling Site	Volume Sampled, gal.	Bacteria (a) Total Plate Count or (b) Total Coliform/100 ml[†]	Total Chlorine, mg/l	Viruses Recovered Plaque-Forming Units BGM[*]	Viruses Recovered Plaque-Forming Units PMK[**]
June						
3	F - Distribution System	100	(a)(b) 0	1.3	0	1
4	B - Tributary below Sewage Treatment Plants	81	(b) 7.0×10^3	0	0	8
5	E - Distribution System	100	(a)(b) 0	1.3	0	4
July						
8	C - Raw Water, Water Treatment Plant	100	(b) 1.3×10^2	0	0	2
23	D - Finished Water, Water Treatment Plant	100	(a)(b) 0	1.4	2	0
August						
18	D - Finished Water Water Treatment Plant	100	(a)(b) 0	1.7	2	1
19	A - Tributary Above Sewage Treatment Plants	91	(b) 7.9×10^3	0	1	1

[†] Most Probable Number

[*] Buffalo Green Monkey Cells

[**] Primary Monkey Kidney Cells

TABLE 8. RESULTS OF THE REPRODUCTIVE CAPACITY TEMPERATURE MARKER TESTS PERFORMED ON OCCOQUAN ISOLATES

Isolation Date	Isolation Site	r - Values[a]	Relative Temperature Factor (R)[aa]
June 4	B	$9.0 \times 10^3/1.3 \times 10^6 = 0.007$	0.9
	B	$6.0 \times 10^3/1.0 \times 10^6 = 0.007$	0.8
	B	$2.3 \times 10^3/1.0 \times 10^6 = 0.003$	0.3
	B	$3.8 \times 10^4/1.1 \times 10^6 = 0.035$	4.6
June 5	E	$1.2 \times 10^5/1.2 \times 10^6 = 0.100$	13.2
	E	$2.3 \times 10^4/5.9 \times 10^5 = 0.039$	5.3
July 8	C	$0\ /9.0 \times 10^5 = 0$	0
July 23	D	$6.5 \times 10^4/1.1 \times 10^6 = 0.062$	9.3
	D	$6.5 \times 10^4/7.0 \times 10^5 = 0.093$	13.9
	D	$1.2 \times 10^5/3.1 \times 10^5 = 0.039$	5.8
August 18	D (BGM cells)	$1.0 \times 10^4/6.5 \times 10^5 = 0.015$	2.2
	D (PMK cells)	$3.0 \times 10^4/1.0 \times 10^6 = 0.030$	4.5
	D (PMK cells)	$9.0 \times 10^4/3.0 \times 10^6 = 0.031$	4.6
August 19	A	$5.0 \times 10^3/2.7 \times 10^6 = 0.002$	0.3
Controls	LSc-1 (UMN)	$0\ /7.5 \times 10^4 = 0$	0
	Mahoney	$4.4 \times 10^6/6.0 \times 10^1 = 0.74$	100.0
	LSc-1 (Carborundum)	$0\ /1.2 \times 10^6 = 0$	0
	Virulent: Mahoney	$2.8 \times 10^6/4.2 \times 10^6 = 0.67$	100.0

[a] $r = \frac{\text{Plaque forming units after 18 hours @ 40°C}}{\text{Plaque forming units after 18 hours @ 37°C}}$

[aa] $R = \frac{X_r - A_o r}{V_o r - A_o r} \times 100$ where: X_r = r-value of unknown, $A_o r$ = r-value of avirulent control (LSc-1) ± 0.0
$V_o r$ = r-value of virulent control (Mahoney) ±0.67

TABLE 9. RESULTS OF SEEDED-VIRUS RECOVERY EXPERIMENTS

Date 1975	Site	Sample Volume Gallons	Water Temperature °F	Viruses, PFU					
				Polio		Reo		Adeno	
				Seed	Recovery	Seed	Recovery	Seed	Recovery
May 21	A	84	88	-	-	-	-	5.6×10^5	0
21	A	75	96	Lost	Lost	Lost	Lost	-	-
23	F	95	85	-	-	-	-	6.5×10^5	8.3×10^3 (1.4%)
23	F	100	85	3.3×10^5	2.4×10^5 (73%)	3.9×10^5	0	-	-
24	C	95	92	-	-	-	-	6.0×10^5	1.5×10^5 (25%)
27*	D	85	92	-	-	-	-	3.3×10^5	0
27	D	100	95	4.0×10^5	4.7×10^5 (118%)	4.7×10^5	0	-	-
28	E	96	89	-	-	-	-	3.3×10^5	1.3×10^4 (3.9%)
28	E	100	94	3.9×10^5	3.2×10^5 (82%)	4.5×10^5	0	-	-
29	C	100	89	4.5×10^5	7.2×10^4 (16%)	5.3×10^5	0	-	-
August 28	B	50	89	-	-	-	-	1.0×10^6	8.8×10^3 (0.9%)
28	B	50	89	**	0	**	0	-	-

* Four samples of concentrator effluent were collected and analyzed on this date; all were negative.

** Questionable.

OCCOQUAN-II

Environmental Monitoring: The Carborundum Company

During Occoquan-II, a total of 76 environmental samples were taken from June 17, 1976, through March 25, 1977, including two at the Northside Sewage Treatment Plant (STP), and from these, a total of 97 concentrates (from adsorbing and clarifying filters) were prepared and assayed for viruses. This phase of the project was coded "2-A," a designation which will be used in the discussion section. The majority (55, 72 percent) were taken from finished-water sites. Viruses were recovered on one occasion each at Sites A, B, C, and E and at both sampling points at the STP. A summary of the data relative to these recoveries, exclusive of those at the STP, is given in Table 10.

Note from Table 10 that three of the four virus recoveries were from raw water. The one occasion when an isolate (Polio 1) was reported in a sample taken from finished water (Site E) occurred on a day when viruses were recovered from the rectal swab of The Carborundum Company's field technician. Complete field and laboratory information concerning all the environmental samples is presented in Appendix C, Table C-1. It should be pointed out that no viruses were recovered from the open reservoir (Site H) in Alexandria on two sampling occasions and only once out of seventeen occasions from the raw water used by FCWA even though both the clarifying filters and adsorbing filters were eluted each time. However, a single virus was recovered each time a sample was taken in Bull Run (twice), once above and once below the point where the last of the STP effluents were entering at the time of sampling.

Comparative Studies: Environmental Sampling and Analysis

Tables 11 and 12 present an analysis of the data reported by both the EPA and The Carborundum Company for the samples they analyzed after the sampling event at the Northside STP in October, 1976. Personnel at each laboratory sent the others a portion of the concentrates which they had prepared after they had sampled the unchlorinated influent and chlorinated effluent to the lagoon. The Carborundum Company prepared concentrates from both their adsorbing and clarifying filters (Table 11), and EPA prepared one concentrate from their adsorbing filter (Table 12). As can be seen from both the tables, the EPA group reported higher virus recoveries whether the concentrate they analyzed was their own or The Carborundum Company's. However, from these data it appears that The Carborundum Company's concentrator more effectively concentrated viruses. [For example, compare EPA concentrated/EPA analyzed (Table 12) value of 268.6 PFU per gallon with Carborundum concentrated/EPA analyzed (Table 11) value of 3273 PFU per gallon.]

The EPA group sampled eleven additional times from Sites C, D, E, and F at the same time the Carborundum group sampled. As has been described previously, each group sent the other a portion of their respective concentrates to be assayed for virus. Table 13 lists the sites from which the samples were taken and the results of The Carborundum Company's analyses of EPA's concentrates. Table 14 shows EPA's results. Note that both laboratories reported finding no viruses in EPA's concentrates. The

TABLE 10. LABORATORY AND FIELD DATA ASSOCIATED WITH ENVIRONMENTAL SAMPLES ON DAYS WHEN VIRUSES WERE RECOVERED AT SITES A,B,C, AND E, OCCOQUAN-II JUNE 17, 1976 - MARCH 25, 1977

Date	Site	Volume, gal.	Duration, hr.	UNH Lab No.	Assay Results		Bacterial Density		Comments
					PFU	Type	MPN/100 ml	TPC/ml	
9/2/76	E (Finished Water)	102	1.9	810-A	1	Polio 1	Total:<3 Fecal: 0	0	1) Carborundum field engineer's rectal swab was positive: 6 Polio-1 & 1 Coxsackie B-4. 2) Chlorine residual in water at time of sampling: 2.3mg/l(free).
10/11/76	C (FCWA raw water)	48	2.8	900-C	1	Polio 2	Total:>2400 Fecal:5400	4.0×10^4	Recovered from clarifying filter. High turbidity water this date.
10/12/76	B (Bull Run Below STP's)	79	3.0	903-C	1	Polio 1	Total>900 Fecal:630	2.0×10^3	Recovered from clarifying filter. Low turbidity on this date.
10/15/76	A (Bull Run above STP's)	100	3.3	908-C	1	Polio 1	Total: 2800 Fecal: 220	2.5×10^3	Recovered from adsorbing filter. Low turbidity on this date.

TABLE 11. RESULTS OF COMPARATIVE VIRUS SAMPLING PROGRAM, PHASE 2-A, INVOLVING CONCENTRATES OF SAMPLES TAKEN FROM NORTHSIDE SEWAGE TREATMENT PLANT BY CARBORUNDUM AND ASSAYED IN THE LABORATORIES OF BOTH THE CARBORUNDUM COMPANY (UNIV. NEW HAMPSHIRE) AND THE EPA'S HEALTH EFFECTS RESEARCH LABS (CINCINNATI)

Sampling Date	Sampling Site Northside Sewage Treatment Plant	Sample Volume, gal	Final Concentrate, ml.			Results of Virus Assays, Plaque Forming Units (PFU)							
			Total	Assayed		PFU Observed		PFU per ml Conc.		[e]Calc. PFU-Entire Conc.		[f]Calc. PFU per gal. sample	
				Carbo	EPA	Carbo	EPA	Carbo	EPA	Carbo	EPA	Carbo	EPA
Oct. 13, 1976	[a]Lagoon	2.2	[c]C:51.8	12.9	23	9	506	0.70	22	36	1140	16.4	518
	Influent		[c]A:56.0	7.0	28	38	3080	5.43	110	304	6160	138	3273
						[d]S: 47	3586			[d]S:340	[d]S:7200		
Oct. 14, 1976	[b]Effluent, Chlorine Contact Basin	50	[c]C: 70	23.8	24	11	-	0.46	-	32.4	-	0.7	-
			[c]A:123	19.1	40	49	2560	2.57	64	315.6	7872	6.3	157.4
						[d]S 60	2560			[d]S:239.0	[d]S:7872		

[a] Lagoon following secondary clarifier

[b] Chlorine applied after discharge from lagoon. Total residual = 2.4 mg/l, free = 2.3 mg/l

[c] C= clarifier filter, A= Adsorbing filter on Aquella^{tm} Virus Concentrator.

[d] S= sum of data for clarifying and adsorbing filters

[e] Calculated as follows: PFU per ml. concentrate x Total Concentrate Volume

[f] Calculated as follows: Calculated PFU for Entire Concentrate ÷ Sample Volume, gallons

TABLE 12. RESULTS OF COMPARATIVE VIRUS SAMPLING PROGRAM, PHASE 2-A, INVOLVING CONCENTRATES OF SAMPLES TAKEN FROM NORTHSIDE SEWAGE TREATMENT PLANT BY THE VIRUS RESEARCH GROUP AT EPA'S HEALTH EFFECTS RESEARCH LABS (CINCINNATI) AND ASSAYED IN THE LABORATORIES OF BOTH THE EPA AND THE CARBORUNDUM COMPANY (UNIV. OF NEW HAMPSHIRE)

Sampling Date	Sampling Site Northside Sewage Treatment Plant	Sample Volume, gal	[a]Final Concentrate, ml.			[a]Results of Virus Assays, Plaque Forming Units (PFU)							
			Total	Assayed		PFU Observed		PFU per ml Conc.		[d]Calc.PFU-Entire Con.		[e]Calc. PFU per gal. sample	
				Carbo	EPA	Carbo	EPA	Carbo	EPA	Carbo	EPA	Carbo	EPA
Oct. 13, 1976	[b]Lagoon Influent	8.5	24	5.8	12	90	1128	15.2	94	372.4	2256	43.8	268.6
Oct. 14, 1976	[c]Effluent, Chlorine Contact Basin	100	28.8	7.3	14.4	14	403	1.9	28	55	806	0.55	8.1

[a] Only the concentrate obtained from the adsorbing filter of EPA's Virus Concentrator was used.

[b] Lagoon following secondary clarifier

[c] Chlorine applied after discharge from lagoon. Total residual = 2.4 mg/l, free = 2.3 mg/l

[d] Calculated as follows: PFU per ml. concentrate x Total Concentrate Volume

[e] Calculated as follows: Calculated PFU for Entire Concentrate ÷ Sample Volume, gallons

TABLE 13. RESULTS REPORTED BY THE CARBORUNDUM COMPANY FOR ASSAYS OF EPA/HERL CONCENTRATES TAKEN DURING PHASE 2-A IN COMPARATIVE SAMPLING AND ANALYSIS EXPERIMENTS, JULY 1976.

Sampling Date	Site	UNH Lab No.	EPA[a] Lab No.	Assay Date (UNH)	Viruses, PFU[b] BGM[c]	Viruses, PFU[b] PMK[d]
July 13	D	763	180	8/25	0	0
14	E	764	181	8/25	0	0
15	C	765	182	8/25	0	0
16	D	766	183	8/25	0	0
19	F	767	184	8/25	0	0
20	E	768	185	8/25	0	0
21	F	769	186	8/25	0	0
22	C	770	187	8/25	0	0
23	D	771	188	8/25	0	0
27	F	772	189	8/25	0	0
28	C	773	190	8/25	0	0

[a] EPA reported no recoveries of virus for these samples

[b] PFU = plaque-forming units

[c] Buffalo Green Monkey Cells

[d] Primary Monkey Kidney Cells

TABLE 14. RESULTS REPORTED BY EPA FOR ASSAYS OF LIQUID-OVERLAY CONCENTRATES PREPARED BY EPA AND THE CARBORUNDUM COMPANY'S VIRUS GROUPS DURING PHASE 2-A IN COMPARATIVE SAMPLING AND ANALYSIS EXPERIMENTS, JULY, 1976

		Viruses, PFU, Recovered From Three Cell Types[a]					
		Carborundum Samples			EPA Samples		
Sampling Date	Site	RD	BGM	PMK	RD	BGM	PMK
7/13/76	D	0	0	0	0	0	0
7/14/76	E	0	0	0	0	0	0
7/15/76	C-a	0	0	0	[b]0	[b]0	[b]0
	C-c	0	0	0			
7/16/76	D	0	0	0	0	0	0
7/19/76	F	0	0	0	0	0	0
7/20/76	E	0	0	0	0	0	0
7/21/76	F	0	0	0	0	0	0
7/22/76	C-a	0	0	0	[b]0	[b]0	[b]0
	C-c	0	0	0			
7/23/76	D	0	0	0	0	0	0
7/26/76	E	0	0	0	0	0	0
7/27/76	F	0	0	0	0	0	0
7/28/76	C-a	0	0	0	[b]0	[b]0	[b]0
	C-c	0	0	0			

[a] Cells observed for two passages, 14 days each

[b] Clarifier and adsorbent filters eluted and assayed together

supplemental data pertaining to water quality on these dates and the results of Carborundum analyses of their own concentrates (all negative) are found in Appendix C.

ADDITIONAL FIELD STUDIES

Studies Associated With Water Treatment Plant Filters

The results of these studies, designated as phase 1-B for future discussion, are presented in Table 15. As can be seen, the chlorine residuals in all samples were quite high during the tests, the lowest being 1.2 mg/1 in one of the backwash water samples. No viruses were recovered during any of the tests by either EPA or The Carborundum Company's assay laboratories.

Study of the Filter Media

No viruses were recovered from either of the two samples of anthracite (UNH Numbers 1731 and 1770, collected on August 3 and September 6, 1977, respectively, and analyzed on October 4, 1977). The EPA viral assay laboratory reported finding no viruses in the concentrates they received (195 and 100 ml, respectively).

COMPARATIVE STUDY WITH CODED, SEED-VIRUS SAMPLES

The results of the first twenty-five virus-recovery studies are given in Table 16. The number of PFU's in each sample were known only after the samples were decoded once all data had been reported. The numbers in the column entitled "PFU Given to Each Group" were based on the assumptions that the contents of each coded vial had been divided equally (after dilution) between each of the field teams and that any viruses present were monodispersed. Data in the column entitled "Fraction of Total PFU Actually Used by Group" are the fractions of the initial thiosulfate volumes which were injected into the pasteurized water by the proportioning pumps during the first twenty-five experiments.

The virus recoveries by each group, expressed as a percentage, were calculated on the basis of the initial titers determined by CSHD, and an assumption was made that the numbers of plaques recovered by the two assay laboratories would have been double those reported had the entire volume of the final concentrate been analyzed. Recall that each laboratory analyzed one-half of each concentrate; therefore, the data (PFU) they reported were multiplied by 2.0 before the percent recoveries were calculated. On two occasions, (tests numbered 2 and 12 in Table 16) the EPA laboratory reported virus types which were not those provided by CSHD. These are reported in the table as "contaminants." No contaminants were reported by The Carborundum Company, but they picked only one representative plaque from each cell sheet. The EPA laboratory, on the other hand, typed all of them, except when the titer was greater than 10^4 PFU's (test 1).

Table 17 presents the virus recoveries reported by each laboratory, both in PFU's and as percent recoveries, for the experiments in which the concentrates were diluted in the large reservoir. The calculated percent

TABLE 15. PHYSICAL, CHEMICAL, AND BIOLOGICAL DATA COLLECTED DURING SPECIAL STUDIES (PHASE 1-B) TO ATTEMPT ENTERIC VIRUS RECOVERIES FROM FINISHED, FILTERED WATER IMMEDIATELY PRECEDING AND FOLLOWING FILTER BACKWASH AND FROM THE BACKWASH WATER ITSELF IN THE PIPE GALLERY OF THE NEW LORTON FACILITY, FAIRFAX COUNTY WATER AUTHORITY, JUNE, 1977

			Sampling Observations			Water Quality Data				Bacterial Data		Assay Laboratory Data		
								Chlorine Residual mg/1						
Sampling Date	Sample Description	Filter Head Loss & Time in Operation	Begin Time	Duration hr	Volume Sampled gal	pH @ Time	Temp. °C	Total	Free	MPN/100 ml	TPC/ml	[a]No.	Date	[b]PFU
June 2	Backwash Filter No.3 (Iron floc)	9.5 ft 45 hr	10:34 am	2.0	32	7.4@10:20 7.6@12:44	20.5 21.5	c	c	<3	0	1509-a 1510-c	6/29 6/29	0 0
June 3	Backwash Filter No.4 (Iron floc)	7.0 ft 28.5 hr	9:45 am	1.6	42	7.7@9:45 7.2@11.55	21.0 21.5	c	c	<3	0	1511-a 1512-c	6/29 6/29	0 0
June 6	Pre-Backwash Filter No.1	7.5 ft 24.5 hr	9:10 am	1.5	100	7.6@8:50 7.6@10:40	21.0 21.0	2.7 3.1	2.4 2.9	<3	0	1513-a	6/29	0
	Post-Backwash Filter No.1	—	12:35 pm	1.5	104	7.6@12:05 7.6@2:05	21.0 21.0	3.2 3.1	2.7 2.7	<3	0	1514-a	6/29	0
June 7	Pre-Backwash Filter No.4	8.1 ft 42 hr	9:19 am	1.6	100	7.6@9:10 7.7@10:55	21.0 21.0	2.3 2.3	2.1 2.1	<3	0	1515-a	6/29	0
	Backwash Filter No.4 (Iron Floc)	8.1 ft 44 hr	11:58 am	2.0	40	7.6@11:45 7.7@1:59	21.0 21.0	[c]3.0 2.9	[c]2.2 1.4	<3	0	1516-a	6/29	0
	Post-Backwash Filter No.4	—	12:20 pm	1.7	100	7.6@12:10 7.5@2:10	21.0 21.0	2.3 3.0	2.0 2.6	<3	0	1518-a	6/29	0
June 10	Backwash Filter No.4 (Iron Floc)	6.0 ft 45 hr	9:50 am	1.7	39	7.4@9:30 7.5@11:05	20.5 21.0	2.5 1.7	1.2 1.2	<3	0	1517-c	6/29	0

[a] UNH numbers: "a" designation is for the adsorbing filter eluate, "c" is for the clarifying filter eluate

[b] Plaque-forming units, BGM and FMK cell lines

[c] Interference with the DPD procedure was noted on June 2 and June 3. On June 7, samples were analyzed by amperometric titration

TABLE 16. VIRUS RECOVERIES BY THE CARBORUNDUM COMPANY AND THE ENVIRONMENTAL PROTECTION AGENCY'S VIRUS GROUPS DURING THE COMPARATIVE SAMPLING-AND-ASSAY STUDY WITH CODED SAMPLES SUPPLIED BY CALIFORNIA STATE HEALTH DEPARTMENT. CONCENTRATES DIVIDED EQUALLY AND DILUTED IN THIOSULFATE RESERVOIRS OF THE RESPECTIVE GROUPS

Test No.	[a]PFU & (Virus) Given To Each Group	Fraction of Total PFU Actually Used by Group		Total Number of PFU Actually Used by Group		Actual Recoveries, PFU & Calculated Efficiency, Per Cent[b]			
						Carborundum-Concentrated		EPA-Concentrated	
		Carborundum	EPA	Carborundum	EPA	Carb.-Analyzed	EPA-Analyzed	Carb.-Analyzed	EPA-Analyzed
1	1.45×10^6 (P-2)	0.63	0.83	0.92×10^6	1.2×10^6	2.2×10^4, 4%	*TNTC	4.6×10^4, 8%	*TNTC
2	150 (P-1)	0.56	0.83	84	124	5, 12%	6, 10%	1, 2%	[c]Contam.
3	0	0.60	0.82	0	0	0	0	0	0
4	150 (P-1)	0.26	0.82	38	123	1, 5%	14, 22%	8, 42%	30, 49%
5	26.5 (P-1)	0.63	0.84	16	22	0	3, 27%	1, 12%	6, 54%
6	26.5 (P-1)	0.60	0.83	15	22	0	3, 27%	2, 27%	4, 36%
7	0	0.62	0.83	0	0	0	0	0	0
8	150 (P-1)	0.69	0.87	103	130	4, 8%	15, 10%	5, 10%	49, 75%
9	150 (P-1)	0.58	0.85	86	127	0	5, 8%	0	31, 49%
10	0	0.68	0.85	0	0	0	0	0	0
11	26.5 (P-1)	0.58	0.73	15	19	0	1, 10%	0,	7, 74%
12	125 (P-2)	0.62	0.79	77	98	2, 6%	5, 10%	6, 16%	[d]Contam.
13	0	0.54	0.89	0	0	0	0	0	0
14	0	0.79	0.88	0	0	0	0	0	0
15	0	0.51	0.86	0	0	0	0	0	0
16	0	0.57	0.86	0	0	0	0	0	0
17	26.5 (P-1)	0.60	0.86	15	22	0	0	0	8,73%
18	0	0.70	0.85	0	0	0	0	0	0
19	0	0.54	0.87	0	0	0	0	0	0
20	150 (P-1)	0.72	0.87	108	130	3, 6%	14, 22%	15, 28%	63, 97%
21	150 (P-1)	0.56	0.90	83	135	9, 22%	11, 16%	15, 36%	33, 49%

--continued--

TABLE 16. Continued

Test No.	[a]PFU & (Virus) Given To Each Group	Fraction of Total PFU Actually Used by Group		Total Number of PFU Actually Used by Group		Actual Recoveries, PFU, & Calculated Efficiency, Per Cent[b]			
						Carborundum-Concentrated		EPA-Concentrated	
		Carborundum	EPA	Carborundum	EPA	Carb.-Analyzed	EPA-Analyzed	Carb.-Analyzed	EPA-Analyzed
22	26.5 (P-1)	0.67	0.93	17	24	0	1, 8%	2, 24%	3, 25%
23	150 (P-1)	0.56	0.88	84	132	2, 5%	6, 9%	8, 19%	38, 58%
24	150 (P-1)	0.58	0.97	87	144	5, 11%	16, 22%	9, 21%	25, 35%
25	0	0.47	0.88	0	0	0	0	0	0

[a] Plaque-forming units: number present in entire contents divided equally among participants. P-1 = polio 1; P-2 = polio 2

[b] Per cent efficiency calculated as follows: (PFU recovered X 2)/PFU Actually Used (CSHD initial titer) X 100

[c] Contamination in EPA-analyzed sample: recovered 13 Polio 1 and 11 Polio 2. (Sample was Polio 1). Plaque total = 38.

[d] Contamination in EPA-analyzed sample: recovered 18 Polio 2 and 5 Polio 1 (Sample was Polio 2; Recovery=37%)

[*] TNTC= too numerous to count at dilution used for assay.

TABLE 17. VIRUS RECOVERIES BY THE CARBORUNDUM COMPANY AND THE ENVIRONMENTAL PROTECTION AGENCY'S VIRUS GROUPS DURING THE COMPARATIVE SAMPLING-AND-ASSAY STUDY WITH CODED SAMPLES PROVIDED BY CALIFORNIA STATE HEALTH DEPARTMENT CONCENTRATES ADDED TO 250 GALLONS OF PASTEURIZED WATER

Test No.	Virus Conc.,[a] [b]PFU/100 gal. & Type	Actual Recoveries, PFU & Calculated Efficiency, Per Cent[c]			
		Carborundum - Concentrated		EPA - Concentrated	
		Carb-Analyzed	EPA Analyzed	Carb-Analyzed	EPA Analyzed
26	120 Polio 1	2,3%	14,23%	0	1,2%
27	21.2 Polio 1	0	0	0	0
28	1.16×10^6 Polio 2	1.7×10^4 3%	2.07×10^4 4%	2.9×10^4 5%	2.5×10^4 4%
29	Blank	0	0	0	0
30	120 Polio 1	0	1,2%	5,8%	4,7%
31	120 Polio 1	3,5%	3,5%	0	11,18%
32	21.2 Polio 1	2,19%	0	1,9%	1,9%
33	21.2 Polio 1	3,28%	[d]contaminated	0	0
34	100 Polio 2	1,2%	6,12%	5,10%	2,4%
35	120 Polio 1	1,2%	4,7%	5,8%	20,33%

[a] Each team passed 100 gallons through its respective concentrator

[b] Plaque forming units

[c] Percent calculated as follows: (PFU recovered x 2) divided by (PFU/100 gal) x 100. PFU/100 gallon figure based on CSHD initial titers.

[d] 1 Polio-3 isolate

recoveries were based on the same assumptions as those in Table 16. Both laboratories typed all viruses recovered except when more than 10^4 PFU's were recovered (test 28).

The original numbers assigned to the vials sent by CSHD and their respective code and recode numbers assigned at FCWA are tabulated in Appendix Table D-1.

Table 18 shows the results reported for the sets of vials stored at FCWA during the course of the study and analyzed directly by EPA and Carborundum at the end of the study. These analyses were planned so that any deterioration in virus titer due to storage, shipping, etc., could be detected. As can be seen, the numbers recovered were less than 40 percent in all vials and below 15 percent in the majority (based on the initial titers reported by CSHD). In November, 1977, fourteen months after the initial titers were first determined, the CSHD laboratory reanalyzed samples of the concentrates they had retained in their laboratory. The titers had decreased from 61 to 79 percent (67 percent average) of their original level. The implications of this observation will be discussed in the discussion section (Section 6).

PERSONNEL SURVEILLANCE

The results of the analyses of throat and rectal swabs for the field personnel and others who entered the Carborundum van during a series of experiments are tabulated in Appendix Table E-1. The recovery of viruses from one rectal swab on a single occasion has previously been mentioned. No other analyses of swabs were positive. EPA's swab data are presented in Appendix Table E-2.

TABLE 18. VIRUS TITERS REPORTED AFTER DIRECT ANALYSES OF CODED VIAL CONTENTS STORED AT FCWA DURING THE COMPARATIVE SAMPLING-AND-ANALYSIS PROGRAM (PERCENT RECOVERIES BASED ON CSHD INITIAL TITERS)

CSHD Lab		Carborundum Lab		EPA Lab	
PFU	TYPE	PFU	% Recovery	PFU	% Recovery
0	Blank	0	0	0	0
-		0	0	0	0
		0	0	0	0
		0	0	0	0
		-	-	0	0
53	Polio-1	8	15	8	15
		6	11	10	19
		7	13	10	19
		6	11	13	25
		6	11	9	17
		$\bar{x}$=6.6	$\bar{x}$=12%	$\bar{x}$=10	$\bar{x}$=19%
250	Polio-2	26	10	93	37
		24	10	63	21
		52	21	86	29
		44	18	81	27
		27	11	92	37
		$\bar{x}$=34.6	$\bar{x}$=14%	$\bar{x}$=83	$\bar{x}$=33%
300	Polio-1	41	14	113	45
		41	14	110	37
		33	11	93	31
		21	7	96	39
		21	7	114	46
		$\bar{x}$=31.4	$\bar{x}$=10%	$\bar{x}$=105.2	$\bar{x}$=35%
2.9×10^6	Polio-2	55500/ml	10	1.82×10^5	6
		61500/ml	11	1.14×10^5	4
		67330/ml	12	2.16×10^5	7
		33500/ml	6	1.72×10^5	6
		28000/ml	5	2.00×10^5	7
		[a] $\bar{x}$=2.5×10^5	$\bar{x}$=8%	$\bar{x}$=1.77×10^5	$\bar{x}$=6%

[a] 5 ml sample: % Recoveries and Means ($\bar{x}$) are based on PFU reported X 5.

SECTION 6

DISCUSSION

OCCOQUAN-1

The report of viruses in finished water during Occoquan-I was the impetus behind most of the activities that comprised the virus portion of Occoquan-II. The first year's report prompted close scrutiny of the Carborundum Company's field- and laboratory procedures--resulting in some changes in the field procedures during Occoquan-II--and was directly responsible for the involvement of EPA's virus-research group in the second year's effort. Never before has a study such as the comparative sampling-and-analysis study with coded samples been reported in the United States. It provided a unique opportunity, not only to compare the specific efficiency and reliability of field and laboratory procedures used by EPA and The Carborundum Company, but also to evaluate the existing, tentative standard method for virus concentration.

The discussion which follows focuses on the reported virus findings during Occoquan-I, the follow-up studies which resulted from these findings, and the studies during Occoquan-II which were planned because of their potential for providing more insight into the interpretation of the first year's results.

Environmental Monitoring

Finished-Water Isolates--

The four finished-water samples from which viruses were isolated during Occoquan-I represents 18 percent of the total finished-water sampling events. The chlorine residuals in the samples exceeded 1.0 mg/l, and coliforms were absent (Table 7). For these reasons, the results were regarded as highly unusual, and prompted much comment and discussion by all parties concerned. While it obviously would have been difficult to determine after-the-fact that the isolates indeed were present in the original 100 gallons of water taken from each site, follow-up investigations involving several parties were made in an attempt to provide some insight into the findings. It should be mentioned that from November, 1974, through September, 1978, 101 samples (100 gallons each) of finished water at FCWA were all negative for viruses. The samples were taken by FCWA and OWMP personnel but were assayed at the Jackson Estuarine Laboratory (JEL) where all Occoquan-I samples were assayed.

The first and most logical investigation was into the nature of the recovered viruses themselves. This investigation involved the rct-marker studies, mentioned previously, and, in addition, the progeny viruses from the original isolates were sent to the Center for Disease

Control in Atlanta for further characterization. Next, the EPA made an inspection of FCWA's facilities to determine whether the routine operating practices there could in any way be conducive to virus contamination. In addition, an EPA virologist visited the JEL to review the laboratory-assay procedures used by those who reported isolating the viruses from the samples. These investigations are discussed in the following paragraphs.

RCT-marker studies--The tests to determine the rct-characteristics of all the natural isolates except the one from Site F (Table 8) showed that none possessed the T+ marker. All of the R values (defined in Table 8) fell within the range (0-30) typical of attenuated strains. However, all but one of the R-values were greater than that calculated for the LSc-1 attenuated strain which was used in the virus-seed experiments during Occoquan-I.

The fact that all but one of the isolates survived at 40°C (T+) is of particular importance because it gives some basis for distinguishing them from seed viruses. Reproduction capacities ranging from 2.5×10^3 to 1.2×10^5 PFU's were recorded for the natural isolates, whereas the LSc-1 strain (control) did not survive. Although the T-marker tests were not performed for the purpose of providing an experimental basis for distinguishing among the isolates (i.e., for identifying each as a distinctly different virus strain), the data do indicate differing growth behavior as a function of temperature. Therefore, some of the isolates appeared to differ from each other as well as from the seed strain (LSc-1). It should be mentioned that virologists differ on the significance of T-marker studies in general as a means of differentiating between polio-virus strains.

Involvement of independent parties--Representatives from EPA's Region III Water Supply Branch and Virginia's State Health Department conducted a site survey of FCWA's three water treatment plants in January, 1976, to determine if there was any obvious operational procedure which might account for the virus recoveries. Their report noted: 1) the high chlorine residuals (average, 2.0-2.5 mg/l free chlorine) and low turbidities (0.1-0.2 units) in finished water during June-August, 1975, when viruses were reported, and 2) the fact that no viruses had been in bimonthly samples collected by FCWA personnel and analyzed at the JEL. It also noted: 1) that the New Lorton plant was not equipped with filter-to-waste lines (the Old Lorton and Occoquan plants were) which required that filters be put in operation immediately after backwash is completed and 2) that filtration rates at the New Lorton Plant were from 5 to 7 gallons per square foot per minute for a brief time after the backwash cycle is initiated when the influent is shut off and the rate controller is opened. In the opinion of the survey team, this was the worst time for a rate increase because floc breakthrough into the finished water is possible under these conditions. This comment was responsible for the special studies during Phase 1-B of Occoquan-II involving attempts to isolate viruses in filter effluent before, during, and after the backwash cycle. The survey team also commented that filter rates exceeding the rated capacity were noted at one or two of the three plants on the days viruses were reported.

In February, 1976, the Chief of the Enterovirus Branch of the

Center for Disease Control (CDC) (Public Health Service, Department of Health, Education, and Welfare) was contacted and requested to examine the Occoquan isolates. The six isolates from Site D (7/23 and 8/18/75) were sent to CDC from UNH, and a report was made in April, 1976. The results are presented in Table 19. The report stated:

"...It is not possible to draw firm conclusions as to the origin of these viruses from these results. The viruses might be of vaccine origin with antigenic characteristics which have 'drifted' to the non-vaccine-like state observed (type 1 is known to change antigenically in this manner during the course of multiplication in the human intestinal tract). On the other hand, these viruses might be 'wild' strains with the rct characteristics observed (about 30 percent of type 1 strains isolated before oral polio vaccine was used, and therefore presumably 'wild' strains, show rct characteristics like those observed)."

It should be noted that neither The Carborundum Company's field technician, any of his immediate family, or either of OWMP's two field technicians who were responsible for the sample concentrations during Occoquan-I had received polio vaccinations during 1975. However, the precaution of routinely taking throat and rectal swabs and storing them in the event a positive virus was found was not observed during Occoquan-I. Therefore, whether one or more of the field personnel were shedding viruses on the days recoveries were reported is purely conjecture.

In February, 1976, an EPA representative from the Cincinnati laboratories visited UNH and the JEL to evaluate laboratory procedures and facilities for handling virus samples. Their report included an overview of these procedures and concluded:

"...none of the procedures (which were) observed would account for the reported isolation of the single type virus from various waters." The writer continued: "The only plausible explanation would seem to be a contribution from a carrier, someone along the line from samplers to laboratory technicians, who might intermittently shed virus and thereby contaminate a sample." The latter statement, of course, is highly speculative, but it strengthened the position that if a contamination had occurred, the assay laboratory most likely was not involved.

In summary, none of the studies succeeded in resolving the issues surrounding these virus isolates. The greatest area of uncertainty was with the actual field procedures used during Occoquan-I when the field crew was not following a routine program of surveillance (swab tests), and stringent precautions to protect the equipment and chemicals from contamination in the field were not being observed. Whereas many arguments can be put forth concerning both sides of the issue, the fact remains that there was no consensus of opinion whether these isolates were really in the drinking water or were present as contaminants. The issue remains today and most likely can never be resolved scientifically.

Reservoir and Tributary Isolates--

Viruses were recovered from Bull Run (Sites A and B) and from the Occoquan Reservoir at Site C on only three occasions, one at each site, during June-August, 1975. Normally, one would expect to recover viruses

TABLE 19. RESULTS OF STRAIN CHARACTERIZATION TESTS PROVIDED BY THE CENTER FOR DISEASE CONTROL FOR THE OCCOQUAN ISOLATES

Isolate	[a]R-Value	Antigenic Characteristic[b]	RCT Characteristic	
			39.5°C	39.9°C
7/23/75				
#1	5.8	NVC	±	-
#2	13.9	NVC	±	-
#3	9.3	NVC	±	-
8/18/75				
#1	4.6	NVC	-	-
#2	2.2	NVC	-	-
#3	4.5	NVC	-	[c]NT

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[a] Determined at UNH, Table 8

[b] NVC = Nonvaccine-like determined by CDC's modified Wecker antigenic differentiation test

[c] Not tested

more frequently from waters that receive treated sewage, especially immediately downstream of the discharges as was Site B, and certainly one would expect more frequent virus recoveries from raw water entering the water treatment plant if finished-water samples from that plant were positive for viruses on several occasions. The failure to recover virus from the natural waters more frequently cannot be explained. It should be noted that during Occoquan-I, the first clarifying filters were never eluted, but from November, 1974 through 1976, both the adsorbing and clarifying filters were eluted and analyzed as part of OWMP's routine monitoring program for viruses. Only twice were viruses reported in raw water samples during that period. Through September 5, 1978, there had been 100 samples taken from Site C (as part of the OWMP) and 167 concentrates from the adsorbing and clarifying filters analyzed. On only three additional occasions, all in 1978, were virus recoveries from Site C reported (Jan. 10--11 reovirus; Jan. 24--1 polio 3; and Oct. 16--1 Echo 7, 2 polio 3). The infrequency of virus recoveries indicates that the impact of sewage treatment discharges and urban runoff on virus levels was not significant. Any potential problem that might have existed would have been further alleviated when the UOSA advanced waste treatment plant went on-line in late June, 1978.

Seeded Samples

The reason for the failure to recover reovirus from seeded samples at any of the six sites during Occoquan-I is not known. As was pointed out, The Carborundum Company has recovered reovirus from natural samples on numerous occasions. (In 1978, eleven reoviruses were recovered at Site C in one sample). However, because seed virus could not be recovered, the only conclusion one can draw is that the desired monitoring for reovirus during Occoquan-I was not accomplished. Recoveries of the adenovirus were erratic (0.9-25%, Table 9), but the numbers of viruses recovered actually were large (10^3-10^5). Thus, the problems which caused the failure to recover reovirus apparently did not interfere with adenovirus recovery (nor with poliovirus either. See Table 9).

OCCOQUAN-II

Environmental Monitoring

Only once was an isolate reported from a finished-water site during Occoquan-II, that being one Polio 1 at Site E (Alexandria, Va.) on September 2, 1976. A rectal swab taken this day also showed the only positive results of any swab examined during the program. Six Polio-1 and one Coxsackie B-4 viral units were recovered. All the polio isolates were shown to be avirulent by T-marker tests. The water-associated isolate and five of the six poliovirus, swab-associated isolates were sent to the CDC for examination. Tests confirmed that all these were nonvaccine-like (Table 20). While the technician did not demonstrate a history of shedding before or after this event (Appendix Table E-1) and while the low numbers of isolates indicate that he was not actively shedding, one still cannot completely rule out the chance, however remote, that the isolate recovered from the finished-water sample was the result of a chance contamination by the field technician.

As was true during Occoquan-I, viruses were recovered very seldom

TABLE 20. ANTIGENIC AND RCT CHARACTERISTICS OF OCCOQUAN-II VIRUS ISOLATES FROM FINISHED WATER AND A RECTAL SWAB

Sample	UNH No.	CDC Data		UNH Data	
		RCT 39.5°C	Antigenic Test	r[a]	R[a]
Finished Water, Site E, 9/2/76	810-3	[c]Neg.	[b]NVC	0.064	0.55
Rectal Swab, 9/2/76	812-4	Neg.	NVC	0.044	0
	812-5	Neg.	NVC	0.048	0
	812-6	Neg.	NVC	0.055	0
	812-7	Neg.	NVC	0.079	1.94
LSc Control				0.058	0
Mahoney Control				1.14	100

[a] See Table 8 (Results Section) for details

[b] Nonvaccine-like

[c] Neg. = Negative

from natural waters during the second year's effort. A series of samples taken the week of October 11, 1976, yielded most of the natural isolates, A single Polio 2 isolate was recovered from the Occoquan Reservoir (raw water (Site C) and a Polio 1 isolate was obtained from Bull Run at Site A above all the STP discharges. Three isolates--one Polio 1 and two Coxsackie B-4--were recovered from Bull Run below the STP discharges (Site B). The isolates were obtained from the clarifying filters used at Sites B and C and from the adsorbing filter at Site A.

The comparative sampling events when both EPA and The Carborundum Company sampled natural waters were nonproductive because no viruses were recovered. The results from the STP samplings served only to demonstrate: 1) that both teams could recover virus from the same location, 2) that Carborundum's sampler was more effective for virus concentration on that date, and 3) that EPA's laboratory analyses resulted in more virus isolations than UNH's.

Additional Field Studies

Viruses which enter a water treatment plant and survive treatment should be concentrated in the floc which either settles in the clarifier or is trapped within the filter. For this reason, the sampling events associated with the filter gallery at FCWA were designed in the belief that the chances for virus recovery there were much greater than at finished-water sites. None were recovered, however, either from the filter underdrains immediately before and following backwash, in the backwash water itself, or from the anthracite filter media. In retrospect, a better opportunity for virus recovery from the backwash water might have been provided had the floc remained in suspension during the concentration step. Of course, less water would have been passed through the concentrator, because the suspended floc would have plugged the clarifying filter, but then, too, the likelihood would be greater that viruses would be trapped within the concentrator. As it was, the floc was allowed to settle in a large container and only the supernatant was passed through the concentrator.

In retrospect, the failure to recover viruses from the anthracite filter media is not particularly surprising. It has been shown at UNH that the efficiency of virus recovery from adsorption sites is inversely related to the time lapse before elution is attempted. Any viruses on the anthracite most likely had been there for several hours before the media was taken from the filter and several days before the media was eluted. A better procedure, it seems, would be to perform on-site elutions rather than attempt to store and transport the media to a laboratory.

Comparative Study With Coded, Seeded-Virus Samples

Relative Efficiencies--

Tables 21 and 22 summarize the data presented earlier in Tables 16 and 17. The recovery percentages given in these tables were based on the original titers reported by CSHD. The EPA virus research group performance (EPA concentration/EPA assay) was obviously superior to that of The Carborundum Company (Carborundum concentration/Carborundum analysis)

TABLE 21. SUMMARY OF VIRUS RECOVERY DATA (BASED ON CALIFORNIA STATE HEALTH DEPARTMENT'S INITIAL TITER) OBTAINED DURING THE COMPARATIVE SAMPLING-AND-ASSAY STUDY IN WHICH VIRUSES WERE INJECTED IN THE THIOSULFATE RESERVOIRS

Concentrator/ Assay Combination	Avg. Recovery ± Std. Deviation, %, For Virus Levels Shown, [a]PFU				
	Blank	26.5 (Polio 1)	125 (Polio 2)	150 (Polio 1)	1.5×10^{6} (Polio 2)
EPA/EPA					
No. of samples	9	5	1	8	1
Avg. Recovery ± Std. Dev.	0	52% ± 22%	36[b]	59% ± 21%[c]	d
Carbo/Carbo					
No. of Samples	9	5	1	8	1
Avg. Recovery ± Std. Dev.	0	0	6%	11% ± 6%	4%
Carbo/EPA					
No. of samples	9	5	1	8	1
Avg. Recovery ± Std. Dev.	0	14% ± 12%	10%	15% ± 6%	d
EPA/Carbo					
No. of Samples	9	5	1	8	1
Avg. Recovery ± Std. Dev.	0	13% ± 13%	16%	20% ± 15%	8%

[a] Virus levels represent the average number of viruses dispersed in the sodium thiosulfate reservoirs of the respective sampling teams. PFU means plaque forming units. Contamination was noted only in two test runs as shown in notes b & c.

[b] Analysis revealed contamination by five polio 1 viruses in a basic polio 2 virus test

[c] Analysis revealed contamination by eleven polio 2 viruses in a basic polio 1 virus test

[d] Average recovery could not be computed because titre noted was too numerous to count at the dilution selected

TABLE 22. SUMMARY OF VIRUS RECOVERY DATA (BASED ON CALIFORNIA STATE HEALTH DEPARTMENT'S INITIAL TITER) OBTAINED DURING THE COMPARATIVE SAMPLING-AND-ANALYSIS STUDY IN WHICH VIRUSES WERE ADDED TO THE 250 GALLON TANK

Concentrator/ Assay Combination	Avg. Recovery % ± Std. Deviation For Virus Conc. Shown, [a]PFU/100 gal				
	Blank	21.2 (Polio 1)	100 (Polio 2)	120 (Polio 1)	1.2 x 10^6 (Polio 2)
EPA/EPA					
No. of Samples	1	3	1	4	1
Avg. Recovery ± Std. Dev.	0	3% (2 negative)	4%	15% ± 15%	4%
Carbo/Carbo					
No. of Samples	1	3	1	4	1
Avg. Recovery ± Std. Dev.	0	16% ± 14% (1 negative)	2%	3% ± 2%	3%
Carbo/EPA					
No. of Samples	1	3[b]	1	4	1
Avg. Recovery ± Std. Dev	0	0	12%	9% ± 9%	4%
EPA/Carbo					
No. of Samples	1	3	1	4	1
Avg. Recovery ± Std. Dev.	0	3% (2 negative)	10%	4% ± 4% (2 negative)	5%

[a] PFU = Plaque forming units. Contamination was noted in only one test run, as is shown in note b.

[b] Analysis revealed contamination by one polio 3 virus in a basic polio 1 virus test.

when the viruses were injected into the sample stream from the thiosulfate reservoir (36%-59% vs. 0%-11%). Furthermore, in every one of the first twenty-five tests, the EPA assay laboratory recovered a higher percentage of the viruses present regardless of who had prepared the concentrate. (Compare EPA/EPA with EPA/Carbo and Carbo/Carbo with Carbo/EPA, Table 20.) The range of mean recoveries when EPA assayed the concentrates was 36-52 percent versus 8-20 percent for Carborundum's data.

When the viruses were added directly to the large reservoir of pasteurized water, both teams' performances were about equal (Table 22), but the efficiencies in each case were notably lower than when the viruses were injected via the thiosulfate reservoir. The reasons for this difference are not known, though one possibility is that the viruses may have been lost by adsorption to the sides of the tank. In these tests, The Carborundum Company's overall performance ranged from 0 to only 18 percent recovery, whereas EPA's performance ranged from 0 to 15 percent.

The percent recoveries expressed in Tables 21 and 22 would be higher if calculated on the basis of CSHD's final titer results. It was unusual that the final titers reported by CSHD for the concentrates, which had been kept frozen throughout the study, were much lower (21-30 percent of the original) than those reported at the beginning of the study. This was surprising because it is generally thought that virus stocks are stable for periods of extended storage. One possible explanation is that the diluent used in preparation of the concentrates was hypertonic and did not contain stabilizing agents such as fetal calf serum. Another is that the monodispersed viruses reassociated into clumps of one or more viruses during storage, and each clump behaved as if it were a single infectious unit during assay.

Figure 8 was prepared by drawing straight lines connecting the initial and final titers reported by CSHD for each of the four levels used in the studies. If the titer reduction is assumed to have occurred linearly, the titers at the times when the experiments were conducted can be estimated. For example, at the time when a vial originally titered at 53 PFU was used in the tests involving dilution of the seed in the thiosulfate reservoir, the titer estimated by interpolation (on the lower line, Figure 8) was only 45 PFU. Thus, the percent recoveries listed in Table 16 corresponding to 53 PFU should be increased by 53/45 or 1.18. Tables 23 and 24 summarize the data for the first 25 and the last 10 experiments, respectively calculated in this manner. The original interpretations of the relative virus-recovery efficiencies of EPA and The Carborundum Company are not altered by this analysis, but the absolute magnitudes of the recoveries increase somewhat. By this analytical procedure the calculated recoveries during the tests in which the viruses were added to the large reservoir would be increased by approximately 30 percent.

The Question of Contamination--

Uninoculated viruses were isolated from sample concentrates of experiments number 2, 11 and 33. The experimental-procedures record indicated that the desired temperature had been reached during the pasteurization of the test water. Therefore, the source of these viral

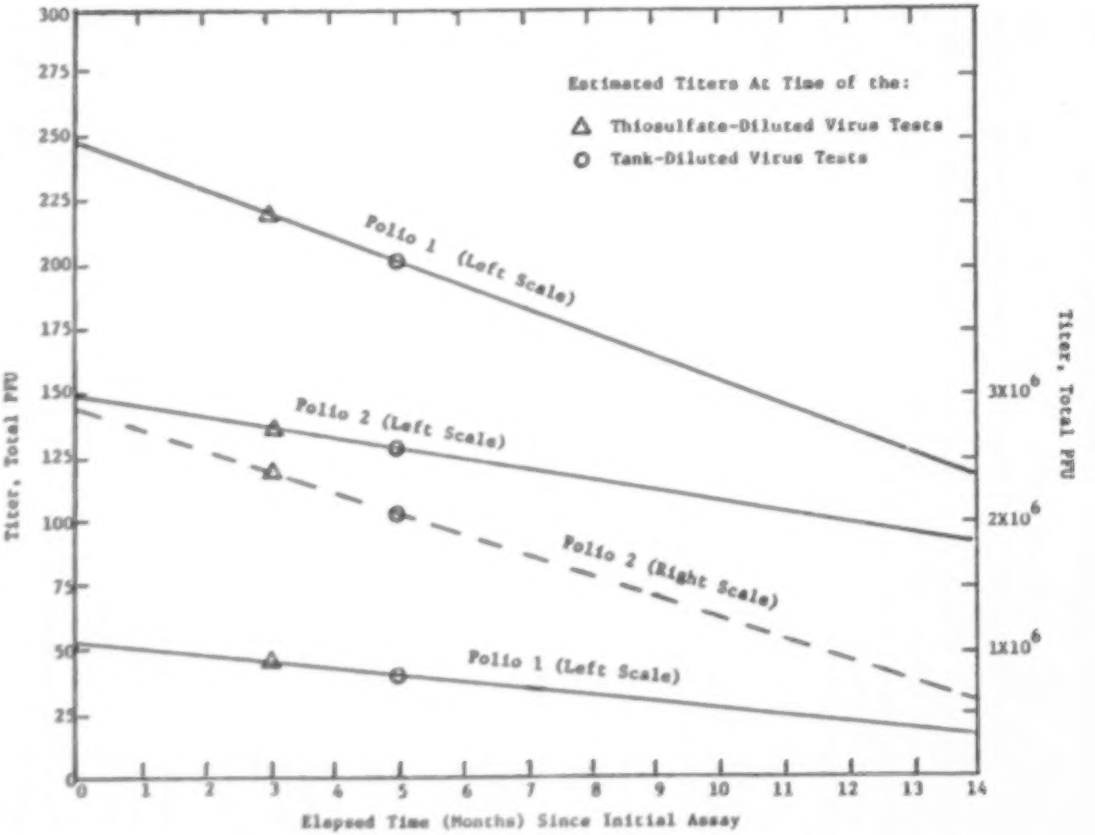

Figure 8. Virus titers in frozen concentrates on four occasions estimated by interpolation and based on an assumption that the observed decrease in titer was linear between the initial and final assay by the California State Health Department laboratory.

TABLE 23. REVISED RECOVERY EFFICIENCIES OF VIRUSES BASED ON AN ASSUMPTION OF A LINEAR, VIRUS-TITER REDUCTION IN FROZEN CONCENTRATES (SAMPLES DILUTED IN THIOSULFATE RESERVOIR)

Concentrator/ Assay Combination	Avg. Recovery ± Std. Deviation for Virus Conc. Shown, [a]PFU			
	26.5 (Polio 1)	125 (Polio 2)	150 (Polio 1)	$1.5 \times 10^{\text{[illegible]}}$ (Polio 2)
EPA/EPA				
No. of Samples	5	1	8	1
Avg. Recovery ± Std. Dev.	62% ± 25%	41%	68% ± 24%	b
[c]Est. titer loss	15%	14%	13%	20%
Carbo/Carbo				
No. of Samples	5	1	8	1
Avg. Recovery ± Std. Dev.	0	8%	9% ± 8%	5%
[c]Est. titer loss	15%	14%	13%	20%
Carbo/EPA				
No. of Samples	5	1	8	1
Avg. Recovery ± Std. Dev.	22% ± 13%	12%	17% ± 7%	b
[c]Est. titer loss	15%	14%	13%	20%
EPA/Carbo				
No. of Samples	5	1	8	1
Avg. Recovery ± Std. Dev.	15% ± 15%	21%	23% ± 18%	10%
[c]Est. titer loss	15%	14%	13%	20%

[a] PFU = Plaque-forming units

[b] Avg. recovery ± standard deviation could not be calculated because the virus titer was too numerous to count at dilution used.

[c] Based on interpolation of data shown in Figure 8.

TABLE 24. REVISED RECOVERY EFFICIENCIES OF VIRUSES BASED ON AN ASSUMPTION OF A LINEAR, VIRUS-TITER REDUCTION IN FROZEN CONCENTRATES (VIRUSES INJECTED INTO 250-GALLON TANK)

Concentrator/ Assay Combination	Avg. Recovery ± Std. Dev. for Virus Conc. Shown, [a]PFU/100 gal			
	21.2 (Polio 1)	100 (Polio 2)	120 (Polio 1)	1.2×10^6 (Polio 2)
EPA/EPA				
No. of Samples	3	1	4	1
Avg. Recovery ± Std. Dev.	4%	5%	19% ± 18%	6%
[b]Est. titer loss	25%	23%	21%	28%
Carbo/Carbo				
No. of Samples	3	1	4	1
Avg. Recovery ± Std. Dev.	21% ± 19%	3%	3% ± 3%	4%
[b]Est. titer loss	25%	23%	21%	28%
Carbo/EPA				
No. of Samples	3	1	4	1
Avg. Recovery ± Std. Dev.	0	16%	12% ± 12%	5%
[b]Est. titer loss	25%	23%	21%	28%
EPA/Carbo				
No. of Samples	3	1	4	1
Avg. Recovery ± Std. Dev.	4%	13%	5% ± 5%	7%
[b]Est. titer loss	25%	23%	21%	28%

[a] PFU = Plaque-forming units.

[b] Based on interpolation of data shown in Figure 8.

isolates could not have been indigenous to the finished drinking water used in these experiments. Also, a test of duplicate vials of the viral inoculum for these experiments revealed that only the appropriate viral type was contained in each vial. Therefore, it must be concluded that the presence of an alien virus in these samples indicates that contamination had occurred at some point during equipment disinfection, sample collection, sample concentration, or sample assessment.

Experiment No. 2 was conducted with poliovirus 1. During the assay/ identification procedure, both laboratories isolated a viral contaminant (poliovirus 2) from their half of the EPA-processed sample concentrate. This finding suggested that contamination had occurred prior to the assessment step.

Experiment No. 12 was conducted with poliovirus 2. The EPA laboratory isolated a contaminant (poliovirus 1) from their half of the sample concentrate that had been processed by EPA. Because the Carborundum laboratory identified the viral type of only one plaque (poliovirus 2) isolated from their half of the EPA-produced sample, it is unknown whether the contaminant was also present there. Therefore, it is impossible to narrow the likely point of contamination to either the collecting, processing, or assessment procedure.

Experiment No. 33 was conducted with poliovirus 1. The EPA laboratory isolated a single plaque, identified as poliovirus 2, from its half of the Carborundum-processed sample. Poliovirus 3 had not been an inoculum in any of the 35 experiments conducted in this study; its isolation unquestionably represented an exogenous contamination.

Because both laboratories had been involved in field surveys of drinking water for viruses, considerable thought and planning had been given previously to the development and use of contamination-prevention procedures. A major objective of this study was to determine the occurrence of viral contamination and, if it occurred, to reveal the weak point(s) in the control procedures.

The experimental design produced 140 subsamples for viral analyses from the 35 experiments. A viral contaminant was revealed in 3 of the 140 subsamples (2%). On two occasions, the contaminant had been the viral type used as the inoculum for the previous experiment. Therefore, this would appear to be a possible source of the contaminant and would implicate the equipment-disinfection procedure of EPA as a weak point in the control procedures. This possibility seems very likely for explaining the contamination of experiment No. 2. The inoculum for experiment No. 1 had been a large number of units of poliovirus 2 (2.4 x 10^6 PFU). The 45 PFU of poliovirus 2 isolated from experiment No. 2 could have represented viral units that remained viable in the equipment after chlorine disinfection. Although this disinfection procedure had been used during an EPA field survey where no contamination was revealed in more than 200 samples, the procedure had never been challenged with viral concentrations greater than 100 PFU.

The previous explanation for the source of the contaminant of experiment No. 12 seems less likely. The viral inoculum for experiment No. 11 had been 40 PFU of poliovirus 1. A total of 16 of the 40 PFU had been recovered from the viral concentrates, 14 by EPA and 2 by Carborundum, leaving 24 viral units that had not been recovered. It seems highly unlikely that these 24 viral units adhered to the equipment's inner surfaces, were not flushed out or inactivated by the acidic chlorine solution, and then were released in a viable form during during the next experimental run where 10 PFU of poliovirus 1 were recovered in addition to 36 PFU of the inoculum (polio 2). This conclusion is supported by the absence of any contamination of samples from 10 experiments conducted with sterile inocula (blanks), many of which followed experiments with higher titers of virus. There is a remote possibility that the EPA sampling equipment was not disinfected subsequent to experiments Nos. 1 and 11. Each were conducted on a Friday prior to weekends on which a change in field personnel took place. The weather was very harsh during the study period, and the discomfort could have resulted in a postponement of the disinfection step. A failure to communicate to incoming personnel the need for equipment disinfection prior to the Monday runs (Nos. 2 and 12) could have occurred. However, the field personnel were all confident that such a break in the disinfection procedure had not occurred.

The most plausible sources of the experiment No. 12 contaminant seemed to be a viral shedder among the field and laboratory personnel or other experiments in the laboratory with the same type of virus. However, no viruses were isolated from the rectal swabs collected from project personnel 15 days before and 31 days after the conducting of experiment No. 12 (Appendix Table E-2). None of the personnel was ill during this period nor had they received a live polio vaccination. In addition, no other projects were ongoing in the laboratory with poliovirus 1. Although this evidence was circumstantial, it did not support the above mentioned possibilities for the origin of the contaminant. Of incidental interest was the finding that the contaminant was equally distributed among the three cell culture flasks used in the assay of the sample, thereby indicating that it had been well mixed into the sample or that contaminated labware had been used in the assay procedure.

The contamination of experiments Nos. 2 and 12 is directly associated with the EPA procedure/personnel. However, the contamination of experiment No. 33 was not as clearly associated with either laboratory. Because poliovirus 3 was not an inoculum for any experiment in this study, its introduction must have been associated with the sample processing (Carborundum) and/or assay (EPA) procedure. However, as in experiment No. 12, an evaluation of personnel and laboratory activity did not reveal a likely source of the contaminant. Further, there were no other findings of poliovirus 3 in the cross-check sampling/analysis conducted by either EPA or Carborundum. Thus, no additional evidence was provided to aid in identifying the source of the contaminant. An additional possibility was also explored, i.e., the erroneous identification of the viral type. A repeat of the neutralization test with a new vial of monospecific antiserum again identified the single isolate from this sample as poliovirus 3.

The failure to obtain any supportive evidence or to advance a tenable hypothesis to explain the source and cause of two of the three sample contaminations is of major concern. Even though this study has been the most extensive one of its type to date, no conclusions as to the necessary control procedures that must be included in a viral field survey can be made. It is obvious that the protocol used for this study was not adequate to prevent contamination. Whether the mechanism of contamination was unique to the circumstances of this study or common to all field studies of this type is unknown. However, it is clear that investigators who are attempting to recover viruses from environmental samples, where positive findings may have major importance, must be extremely diligent in maintaining the integrity of their samples.

.APPENDIX A

Summary of Data Relative To Virus Sampling and Analysis

June - August, 1975

TABLE A-1. SUMMARY OF DATA RELATIVE TO THE VIRUS SAMPLING AND ANALYSIS PROGRAM AS REPORTED BY THE CARBORUNDUM COMPANY, JUNE - AUGUST, 1975

Date	Time	Site	Volume, gal	Duration, hr	Air, °F	Lab No.	Lab Date	Enterovirus, PFU		Reovirus,PFU		Remarks
								BGM	PMK	BGM	PMK	
June												
2	1:35p	A	58	2.6	88	146	7/16	0	0	0	0	
3	11:25a	F	100	1.4	88	126	7/16	0	1	0	0	Rain, polio 1
4	11:29a	B	81	3.8	89	127	7/16	0	8	0	0	polio 1
5	9:00a	E	100	2.0	90	128	7/16	0	4	0	0	polio 1
9	11:32a	A	72	2.1	80	160	8/11	"	"	"	"	Adeno: Negative
						161	8/11	"	"	"	"	
9	2:45p	A	91	3.9	85	129	7/16	0	0	0	0	
10	10:45a	B	71	2.6	85	162	8/11	"	"	"	"	Adeno: Negative
						163	8/11	"	"	"	"	
10	1:55p	B	78	3.1	90	130	7/16	0	0	0	0	
11	9:15a	C	80	2.3	75	164	8/11	"	"	"	"	Adeno: Negative
						165	8/11	"	"	"	"	
11	12:03p	C	99	2.8	-	131	7/16	0	0	0	0	-
12	9:30a	D	100	2.5	85	166	8/11	"	"	"	"	Adeno: Negative
						178	8/11	"	"	"	"	
12	12:59p	D	100	3.0	85	132	7/16	0	0	0	0	
16	11:40a	F	100	1.3	90	171	8/20	"	"	"	"	Adeno: Negative
						172	8/20	"	"	"	"	
16	1:30p	F	100	2.6	89	137	7/24	0	0	0	0	
17	9:10a	E	100	1.8	95	167	8/11	"	"	"	"	Adeno: Negative
						184	9/16	"	"	"	"	
						168	8/11	"	"	"	"	
17	11:45a	E	101	1.8	-	135	7/24	0	0	0	0	
18	9:13a	B	100	2.3	94	169	8/20	"	"	"	"	Adeno: Negative
						170	8/20	"	"	"	"	
18	12:20p	B	77	2.8	-	133	7/24	0	0	0	0	
19	9:20a	A	81	2.9	104	136	7/24	0	0	0	0	
23	9:30a	C	97	2.0	92	173	8/20	"	"	"	"	Adeno: Negative
						181	8/20	"	"	"	"	
23	11:50a	C	80	2.7	96	134	7/24	0	0	0	0	4.3<pH<4.6

(continued)

TABLE A-1. (continued)

Date	Time	Site	Volume, gal	Duration, hr	Air, °F	Lab No.	Lab Date	Enterovirus, PFU BGM	Enterovirus, PFU PMK	Reovirus, PFU BGM	Reovirus, PFU PMK	Remarks
June												
24	10:09a	D	100	1.4	92	179	8/20	0	0	0	0	Adenovirus negative
						180	8/20	0	0	0	0	-
24	12:40a	D	101	3.0	98	148	7/24	0	0	0	0	-
25	9:10a	E	100	1.5	94	174	8/20	0	0	0	0	Adenovirus negative
	11:20a	E	102	1.8	99	149	7/24	0	0	0	0	
26	10:14a	F	100	1.4	83	175	8/20	0	0	0	0	Adenovirus negative
26	12:06p	F	100	1.9	85	150	7/24	0	0	0	0	Open reservoir contributing flow
27	9:25a	A	86	3.3	89	152	8/12	0	0	-	-	-
27	2:15p	B	76	3.8	76	151	7/24	0	0	0	0	Light rain falling
30	9:01a	B	30	2.5	92	176	8/20	0	0	0	0	Heavy rain; water muddy
						185	8/20	0	0	0	0	Adenovirus negative
30	12:00p	B	76	5.0	94	153	8/12	0	0	0	0	Extra filter required
July												
1	10:06a	A	100	2.1	92	177	8/20	0	0	0	0	Adenovirus negative
						186	8/20	0	0	0	0	
1	12:58p	A	84	2.6	102	154	8/12	0	0	0	0	Excess floc present
3	9:20a	E	100	3.0	96	182	8/20	0	0	0	0	Adenovirus negative
7	9:46a	D	100	2.2	91	159	8/12	0	0	-	-	-
8	9:32a	C	100	2.6	93	197	9/11	0	2	-	-	Polio 1 recovered
9	9:11a	B	100	2.6	94	155	8/12	0	0	-	-	
10	10:03a	A	100	3.5	91	198	9/11	0	0	-	-	Pre-Filter added
11	10:21a	F	100	2.0	93	187	9/16	0	0	0	0	Adenovirus negative
14	9:08a	A	54	2.6	75	199	9/11	0	0	-	-	Rain; water muddy
15	9:04a	B	61	3.4	80	200	9/11	0	0	-	-	Rain; water muddy
21	10:01a	C	62	2.1	90	191	9/16	0	0	0	0	Adenovirus negative
21	12:35p	C	81	2.3	98	202	9/11	0	0	0	0	Rain; water turbid
22	10:20a	A	52	2.2	92	188	9/16	0	0	0	0	Adenovirus negative
22	1:00p	A	62	4.5	96	156	8/12	0	0	0	0	Water muddy

(continued)

TABLE A-1. (continued)

Date	Time	Site	Volume, gal	Duration, hr	Air, °F	Lab No.	Lab Date	Enterovirus, PFU		Reovirus, PFU		Remarks
								BGM	PMK	BGM	PMK	
July												
22	4:25p	A	95	2.9	-	192	9/11	0	0	-	-	-
23	9:50a	D	100	1.0	98	-	9/11	0	0	-	-	
23	9:58a	D	95	1.0	96	194	9/11	0	0	-	-	
						201	9/11	1	0	2	0	Poliovirus 1 recovered
23	12:20p	D	90	3.0	98	193	9/11	0	0	-	-	
24	9:10a	B	95	1.8	93	189	-	0	0	0	0	Adenovirus negative
24	12:29p	B	75	3.0	99	157	8/12	0	0	0	0	Water muddy
						195	8/12	0	0	0	0	
28	8:40a	B	85	2.8	82	158	8/12	0	0	-	-	Water muddy & foamy
29	9:16a	A	72	2.9	94	217	9/26	0	0	-	-	Water foamy
August												
4	9:35a	C	100	3.4	98	203	9/26	0	0	-	-	
5	9:06a	A	100	-	93	204	9/26	0	0	-	-	
6	8:24a	B	95	3.6	94	205	-	0	0	-	-	Water foamy,no floc
7	9:09a	D	95	1.8	94	206	9/26	0	0	-	-	
11	8:41a	B	95	2.9	92	207	9/26	0	0	-	-	Water foamy, dirty
12	9:50a	A	86	2.9	96	208	9/26	0	0	-	-	Water turbid
18	9:29a	D	100	1.8	93	209	9/26	2	1	-	-	Poliovirus 1 recovered
19	9:50a	A	91	2.3	92	210	9/26	1	1	-	-	Poliovirus 1 recovered
20	8:50a	B	-	-	94	211	9/26	0	0	-	-	
21	9:00a	C	80	2.6	93	212	9/26	0	0	-	-	Power failure occurred
25	9:18a	A	62	3.2	101	213	9/26	0	0	-	-	Rain; muddy, foamy water
26	9:01a	B	95	2.7	94	214	9/26	0	0	-	-	Water green, foamy
27	9:20a	C	100	3.6	93	2.5	9/26	0	0	-	-	-

APPENDIX B

Supplemental Data Collected on Each Sampling
Date During Occoquan-I, 1975-1976

Key to Table Abbreviations:

OCP = Organochlorine Pesticides
OPI = Organophosphorus Insecticides
CPH = Chlorophenoxy Herbicides
VO = Volatile Organics
HM = Heavy Metals
MPN = Most Probable Number of Coliforms per 100 ml
TPC = Standard Total Plate Count per ml

Key to Observations (Last Column):

1 = Low Turbidity, No Unusual Odor or Color
2 = Moderate Turbidity; No Unusual Odor or Color
3 = High Turbidity; No Unusual Odor or Color
4 = Clear, Colorless, No Unusual Odor

Key to Sites:

A = Catharpin, upper Bull Run Above Treated Sewage Discharges
B = Bull Run Below Treated Sewage Discharges
C = Intake Water at the Fairfax County Water Authority's Water Treatment Facility
D = Finished Water at the Fairfax County Water Authority's Water Treatment Facility
E = Distribution System at the Fairfax County Water Authority's Storage Yard
F = Distribution System in Alexandira, Virginia
G = Distribution System in Dumfries, Virginia

TABLE B-1. PHYSICAL AND CHEMICAL CHARACTERISTICS OF WATER SAMPLED AT ALL SITES (A, B, C, D, E, F)
FOR THE ENTIRE SAMPLING PERIOD - JUNE, 1975 - MAY, 1976
(No data indicates testing not performed)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
5/21/75	Sunny, Hot 85-90°	A	Adeno Virus Seed Run	7.6 @ 10:40pm	21.0	-	-	-	-	1
			Enteric & Reo Virus Seed Run	8.0 @ 2:00pm 8.2 @ 5:10pm	25.0 26.0	- -	- -	- -	- -	1-2 Surface Scum
5/22/75	Partly Cloudy 80-85°	E	Adeno Virus Seed Run	7.4 @ 9:30am 7.3 @ 12:30pm	20.0 20.0	1.3 1.0	1.0 1.0	- -	- -	4
			Enteric & Reo Virus Seed Run	7.6 @ 2:00pm 7.6 @ 3:50pm	20.0 20.0	1.0 1.0	1.0 1.0	- -	- -	4
5/24/75	-	C	Adenovirus Seed Run	7.4 @ 12:50pm 7.2 @ 2:34 pm	21.0 21.0	- -	- -	- -	- -	1
5/27/75	Sunny 80-85°	D	Adeno Virus Seed Run	7.6 @ 10:10am 7.4 @ 11:45 am	22.0	1.5 1.5	1.5 1.5	- -	- -	4
			Enteric & Reo Virus Seed Run	7.6 @ 12:55pm - @ 2:15pm	22.0 22.5	1.5 1.5	1.5 1.5	- -	- -	4
5/28/75	Sunny 80-85°	F	Adeno Virus Seed Run	7.3 @ 11:10am 7.0 @ 12:25pm	22.0 -	0.6 -	0.5 -	- -	- -	4
			Enteric & Reo Virus Seed Run	7.2 @ 2:15pm 7.2 @ 3:24pm	- -	0.7 0.7	0.7 0.7	- -	- -	4
5/29/75	Partly Sunny 80-85°	C	Enteric & Reo Virus Seed Run	7.2 @ 12:04pm 7.2 @ 1:40pm	21.2 21.2	- -	- -	- -	- -	1
6/2/75	Sunny 80-85°	C	All Pesticides V.O., H.M.	6.9 @ 10:30am	22.6	-	-	-	-	1
		D	All Pesticides V.O., H.M.	7.2 @ 10:50am	22.8	1.3	1.3	-	-	4
		A	Enteric & Reo	7.8 @ 1:35pm	25.2	-	-	Total 1100 (310-2500)	-	1
			Virus Run	7.6 @ 3:24pm	26.1	-	-	Fecal 1100 (310-2500)	-	1
6/3/75	Variable Cloudiness 80-85°	E	All Pesticides V.O., H.M.	7.0 @ 9:10am	21.6	1.0	1.0	-	-	4
		F	All Pesticides V.O., H.M.	7.0 @ 10:33am	22.8	1.3	1.3	-	-	4
			Enteric & Reo Virus Run	6.8 @ 11:25am 6.8 @ 12:40pm	23.0 23.0	- -	- -	Total< 2	0	4

(continued)

TABLE D-1.(continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
6/4/75	Sunny 80-85°	A	All Pesticides V.O., T.M.	7.2 @ 9:05am	19.5	-	-	-	-	1
		B	All Pesticides V.O., T.M.	7.0 @ 11:10am	22.6	0	0	-	-	1-2 "Murky" color
			Enteric & Reo Virus Run	7.0 @ 11:25am 7.2 @ 2:10pm	22.6 23.8	0 0	0 0	Total 7000 - (1000-17,000) Fecal 2000		1-2 "Murky" color
6/5/75	Party Cloudy 85-90°	E	Enteric & Reo Virus Run	7.0 @ 9:00am 7.2 @ 10:25am	22.6 22.4	1.3 -	1.3 -	T:<2	0	4
6/9/75	Sunny 75-80°	D	OCP V.O.	7.0 @ 9:30am	23.2	2.0	1.5	-	-	4
		C	OCP V.O.	6.9 @ 10:01am	22.6	-	-	-	-	1
		A	Adeno Virus Run	7.0 @ 11:32am 7.1 @ 1:40pm	18.6 20.6	- -	- -	Total 460 (160-1200) Fecal 50 (65-130)	-	1
			Enteric & Reo Virus Run	7.2 @ 2:41pm 7.4 @ 5:10pm	20.6 21.2	- -	- -	Total 330 (110-930) Fecal 110 (20-250)	-	1
6/10/75	Partly Cloudy 70-75°	A	OCP V.O.	7.2 @ 8:55am	18.6	-	-	-	-	1
		B	OCP V.O.	7.0 @ 10:17am	20.6	<0.1	0	-	-	1
			Adeno Virus Run	7.1 @ 10:45am 7.3 @ 12:45pm	21.6 22.2	<0.1 <0.1	0 0	Total 3300 (1100-9300) Fecal 110 (20-250)	-	1
			Enteric & Reo Virus Run	7.2 @ 1:55pm	22.3	<0.1	0	Total 3300 (1100-9300) Fecal 330 (110-930)	-	1
6/11/75	Cloudy 70-75°	F	OCP V.O.	7.0 @ 2:28pm	24.0	1.5	1.1	-	-	4

(continued)

TABLE D-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
6/11/75	Cloudy 70-75°F	E	GCF V.O.	7.2 @ 3:40pm	22.0	2.3	1.8	-	-	6
		C	Adeno Virus Run	7.0 @ 9:15am 7.0 @ 11:00am	22.4 22.4	- -	- -	Total 350 (130-1000) Fecal 7 (1-17)	-	1
			Enteric & Reo Virus Run	7.0 @ 12:01pm 7.2 @ 1:45pm	22.4 22.3	- -	- -	Total 79 (25-190) Fecal 2 (<0.5-7)	-	1
6/12/75	Rain 70-75°F	D	Adeno Virus Run	7.4 @ 9:25am 7.4 @ 10:47am	22.6 22.6	2.8 2.6	2.4 2.0	Total <2	0	6
			Enteric & Reo Virus Run	7.4 @ 11:40am 7.6 @ 12:55pm	22.6 22.8	2.7 3.0	2.2 2.7	Total <2	0	6
6/16/75	Partly Sunny 85-90°F	C	All Pesticides V.O.	6.65 @ 10:00am	22.6	-	-	-	-	1
		D	All Pesticides V.O.	7.0 @ 10:36am	23.4	2.2	1.8	-	-	6
		F	Adeno Virus Run	6.9 @ 11:30am 7.0 @ 1:05pm	24.2 24.1	2.5 3.1	2.0 2.4	Total <2	0	6
			Enteric & Reo Virus Run	6.9 @ 1:33pm 7.0 @ 3:12pm	24.2 24.1	2.1 1.3	2.1 0.9	Total <2	0	6
6/17/75	Sunny, Hot Humid	A	All Pesticides V.O.	6.9 @ 8:45am	20.6	-	-	-	-	1
		B	All Pesticides V.O.	6.7 @ 1:30pm	25.8	0.3	0.2	-	-	1
		E	Adeno Virus Run	7.2 @ 9:07am 7.2 @ 10:33am	22.7 22.7	1.5 1.6	1.1 1.1	Total <2	0	6
			Enteric & Reo Virus Run	7.2 @ 11:15am 7.4 @ 12:27pm	23.2 23.2	1.2 -	1.0 -	Total <2	0	6
6/18/75	Sunny, Hot Humid	E	[a]GCF V.O.	7.6 @ 1:40pm	22.8	1.1	1.0	-	-	6
		F	GCF V.O.	7.6 @ 3:05pm	25.4	0.7	0.6	-	-	6

[a] Began sampling for V.O. inside restroom due to presence of ketonene vapors at outside tap.

(continued)

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l		Bacteria		Observations
						Total	Free	MPN	TPC	
6/18/75		B	Adeno Virus Run	7.1 @ 9:13am 7.2 @ 11:40am	24.8 25.9	<0.1 <0.1	<0.1 <0	Total 3300 (1100-9300) Fecal 500 (<50-1300)	-	1
			Enteric & Reo Virus Run	7.2 @ 12:20pm 7.0 @ 2:45pm	26.2 27.1	<0.1 -	0 -	Total 1400 (400-3400) Fecal <200	-	turb.more than in morning run
6/19/75	Sunny, Hot 90-95°, Humid	A	Enteric & Reo Virus Run	7.2 @ 9:20am. 7.2 @ 11:15pm	23.2 25.5	- -	- -	Total 1100 (310-2500) Fecal 230 (70-700)	-	1
6/23/75	Sunny, Hot Humid	C	OCP V.O.	7.0 @ 9:05am	23.6	-	-	-	-	1-2, more color than usual
		D	OCP V.O.	7.2 @ 10:15am	24.5	2.5	2.0	-	-	4
		E	OCP V.O.	7.3 @ 2:40pm	25.0	1.7	1.7	-	-	4
		F	OCP V.O.	7.1 @ 3:30pm	26.0	1.4	1.0	-	-	4
		C	Adeno Virus Run	- @ 9:30am 7.0 @ 11:00am	23.6 23.8	- -	- -	Total 8 (1-19) Fecal 5 (<0.5-13)	-	1-2, more color than usual
			Enteric & Reo Virus Run	7.0 @ 11:40am 7.6 @ 1:35pm	23.8 24.0	- -	- -	Total 33 (11-93) Fecal <2	-	1-2, more color than usual
6/24/75	Hazy, Hot Humid	A	OCP V.O.	7.0 @ 8:40am	23.4	-	-	-	-	1
		B	OCP V.O.	7.1 @ 10:00am	25.0	0.2	<0.1	-	-	1
		D	Adeno Virus Run	- @ 10:08am 7.4 @ 11:31am	25.0 24.6	- 1.8	- 1.6	Total <2	0	4
			Enteric & Reo Virus Run	7.4 & 12:30pm 7.2 @ 2:04pm	26.0 27.3	1.8 1.9	1.4 1.8	Total <2	0	4

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
6/25/75	Hazy, Hot 85-90°	E	Adeno Virus Run	7.5 @ 9:16am 7.4 @ 10:34am	25.0 25.0	1.9 1.4	1.7 1.3	Total <2	0	4
			Enteric & Reo Virus Run	7.2 @ 11:16am 7.1 @ 12:30pm	25.0 25.0	1.7 -	1.7 -	Total <2	0	4
6/26/75	Cloudy 75-80° Rain previous evening	F	Adeno Virus Run	7.1 @ 10:14am 7.1 @ 11:26am	26.0 25.9	1.6 2.0	1.5 1.5			filters more color than usual
			Enteric & Reo Virus Run	7.2 @ 12:01pm 6.8 @ 1:25pm	26.0 25.4	2.1 -	1.7 -	Total 2	0	4 filters more color than usual
6/27/75	Cloudy, Showers 75-80°	A	Enteric Virus Run.	7.4 @ 9:25am 7.4 @ 11:45am	22.2 24.6	- -	- -	Total 3300 (1100-9300) Fecal 70 (10-170)	-	1-2 more color than usual
		B	Enteric & Reo Virus Run	7.1 @ 2:10pm 7.1 @ 4:20pm	23.6 25.4	0 0	0 0	Total 33,000 - (11,000-93,000) Fecal 1100 (200-2500)		1-2 more color than usual
6/30/75	Partly Cloudy 80-85° following week-end of heavy rains	B	Adeno Virus Run	7.0 @ 8:58am 6.9 @ 11:05am	22.0 22.4	<0.1 0	0 0	Total 79,000 - (25,000-190,000) Fecal 11,000 (3100-25000)		3
			Enteric & Reo Virus Run	7.1 @ 11:55am 6.8 @ 2:00pm	22.2 23.4	<0.1 -	0 -	Total 49,000 (17,000-130,000) Fecal 4900 (1700-13000)		3
		C	All Pesticides V.O.	6.8 @ 1:50pm	23.8	-	-	-	-	1-2
		D	All Pesticides V.O.	7.1 @ 2:25pm	24.6	2.0	1.8	-	-	4
		E	OCP V.O.	7.3 @ 3:35pm	24.2	1.5	1.2	-	-	4
		F	OCP V.O.	7.6 @ 4:45pm	26.1	1.1	0.7	-	-	4

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual,mg/l		Bacteria		Observations
						Total	Free	MPN	TPC	
7/1/75	Sunny 80-85°	B	All Pesticides V.O.	7.0 @ 8:30am	21.6	<0.1	0	-	-	2-3
		A	All Pesticides V.O.	7.2 @ 9:40am	20.9	-	-	-	-	1-2
			Adeno Virus Run	7.3 @ 10:06am 7.4 @ 12:15pm	21.0 22.4	- -	- -	Total 7000 (2300-17000) Fecal 600 (<50-1500)	-	1-2
			Enteric & Reo Virus Run	7.3 @ 1:00pm 7.9 @ 3:00pm	22.4 23.4	- -	- -	Total 1100 (310-2500) Fecal 110 (20-250)	-	1-2
7/3/75	Sunny 90-95°	E	Adeno Virus Run	7.2 @ 9:20am 7.4 @ 10:40am	24.6 29.6	1.2 0.8	1.0 0.6	Total <2	0	4
7/7/75	Cloudy, Showers	C	OCP V.O., H.M.	7.0 @ 9:00am	24.5	-	-	-	-	1-2, slight musty odor
		D	OCP V.O., H.M.	7.6 @ 9:25am	24.8	1.7	1.6	-	-	4
			Enteric Virus Run	7.6 @ 9:40am 7.1 @ 10:55am	24.8 24.9	1.8 -	1.4 -	Total <2	0	4
		F	OCP, V.O., H.M.	7.2 @ 10:50am	27.0	2.2	1.9	-	-	4
		E	OCP, V.O., H.M.	7.1 @ 12:35pm	23.8	1.1	0.8	-	-	4
7/8/75	Partly Cloudy 80-85°	C	Enteric Virus Run	7.3 @ 9:30am 6.7 @ 11:06am	24.5 24.5	- -	- -	Total 130 (35-300) Fecal 13 (3-31)	-	1-2,black suspended matter appeared at end of run
		A	OCP, V.O., H.M.	7.2 @ 2:20pm	24.8	-	-	-	-	1-2
		B	OCP, V.O., H.M.	7.5 @ 3:05pm	25.5	<0.1	0	-	-	1-2
7/9/75	Partly Cloudy 80-85°	B	Enteric Virus Run	7.3 @ 9:15am 7.3 @ 11:05am	24.4 25.8	<0.1 <0.1	0 0	Total 5000 (6500-13000) Fecal 2000 (<500-7000)	-	1-2
7/10/75	Partly Cloudy 80-85° Showers in Evening	A	Enteric Virus Run	7.5 @ 10:03am 7.4 @ 12:45pm	22.8 24.2	- -	- -	Total 4900 (1700-13000) Fecal 500 (<50-1300)	-	1

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l		Bacteria		Observations
						Total	Free	MPN	TPC	
7/11/75	Partly Sunny 80-85°	F	Adeno Virus Run	7.4 @ 10:21am 7.1 @ 11:41am	26.8 26.8	1.2 1.3	0.9 0.7	Total <2	0	4
7/14/75	Rain 75-80° following weekend of heavy rains	A	Enteric Virus Run	7.1 @ 9:08am 7.2 @ 10:50am	21.0 21.0	- -	- -	Total 79,000 - (25,000-190,000) Fecal 1700 (500-4600)		3
		C	All Pesticides V.O.	6.8 @ 1:00pm	24.4	-	-	-	-	2
		D	All Pesticides V.O.	7.1 @ 1:20pm	25.4	1.6	1.5	-	-	4
		F	OCP, V.O.	7.4 @ 2:10pm	26.0	0.8	0.6	-	-	4
		E	OCP, V.O.	7.2 @ 3:27pm	23.8	0.8	0.7	-	-	4
7/15/75	Variable Cloudiness 80-85°	B	Enteric Virus Run	7.1 @ 9:04am 7.2 @ 10:42am	22.0 23.0	<0.1 0	0 0	Total 33,000 - (11,000-93,000) Fecal 3300 (1100-9300)		2
			All Pesticides V.O.	7.1 @ 9:04am	22.0	<0.1	0	-	-	2
		A	All Pesticides V.O.	7.4 @ 10:00am	21.0	-	-	-	-	2
7/21/75	Hot, Sunny 90-95°	C	OCP, V.O.	7.4 @ 9:20am	24.2	-	-	-	-	1
		D	OCP, V.O.	7.4 @ 10:34am	25.0	1.9	1.7	-	-	4
		F	OCP, V.O.	7.5 @ 1:25pm	26.6	0.5	0.5	-	-	4
		E	OCP, V.O.	7.3 @ 3:00pm	24.6	1.0	0.9	-	-	4
		C	Adeno Virus Run	7.4 @ 10:00am 7.0 @ 11:41am	24.2 24.8	- -	- -	Total 23 (7-70) Fecal 8 (1-19)	-	1 at end of Run 3 yellow color "gamey"smell
			Enteric & Reo Virus Run	7.1 @ 12:30pm	24.8	-	-	Total 33 (11-93) Fecal 2 (<0.5-7)	-	1
7/22/75	Sunny, Hot 90-95°	B	OCP, V.O.	7.2 @ 8:25am	23.8	0	0	-	-	2
		A	OCP, V.O.	7.1 @ 9:42am	23.2	-	-	-	-	1-2

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
7/22/75	Sunny, Hot 90-95°	A	Adeno Virus Run	7.4 @ 10:20am 7.2 @ 11:59am	24.6 26.4	- -	- -	Total 17,000 (4300-49,000) Fecal 500 (<50-1300)	-	1-2
			Enteric-Reo Virus Run	7.4 @ 1:00pm 6.9 @ 4:00pm	26.0 26.8	- -	- -	Total 23,000 (7000-70,000) Fecal 800 (100-1900)	-	1
7/23/75	Sunny, Hot 90-95°	D	Adeno Virus Run	7.4 @ 9:55am 7.5 @ 11:10am	25.7 25.9	1.5 1.5	1.5 1.4	Total <2	0	4
			Enteric Virus Run	7.5 @ 12:20pm	26.2	1.4	1.0	Total <2	0	4
7/24/75	Sunny, Hot Humid 85-90°	B	Adeno Virus Run	7.4 @ 9:10am 7.4 @ 11:00am	25.5 25.5	0 0	0 0	Total 3300 (1100-9300) Fecal 500 (<50-1300)	-	1
			Enteric & Reo Virus Run	7.5 @ 12:24pm 7.1 @ 2:10pm	26.7 27.1	0 0	0 0	Total 4900 (1700-13,000) Fecal <200	-	1
7/28/75	Sunny, Hot Humid	B	Enteric Virus Run	7.4 @ 8:39am 7.4 @ 10:25am	23.4 25.6	0 -	0 -	Total 3300 (1100-9300) Fecal 200 (<50-700)	-	1
		C	All Pesticides V.O.	6.6 @ 12:35pm	25.0	-	-	-	-	1 Color less than usual
		D	All Pesticides V.O.	7.2 @ 1:25pm	27.0	1.8	1.8	-	-	4
		F	OCP, V.O.	7.3 @ 2:26pm	28.0	0.8	0.4	-	-	4
		E	OCP, V.O.	7.1 @ 3:30pm	25.5	0.9	0.8	-	-	4
7/29/75	Sunny, Hot 90-95°	A	Enteric Virus Run	7.5 @ 9:15am 7.4 @ 11:10am	23.0 24.0	- -	- -	Total 1700 (500-4600) Fecal 200 (<50-700)	-	1
			All Pesticides V.O.	7.4 @ 8:26am	23.0	-	-	-	-	1
		B	All Pesticides V.O.	7.1 @ 12:35pm	27.0	<0.1	0	-	-	1

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature oC	Chlorine Residual, mg/l		Bacteria		Observations
						Total	Free	MPN	TPC	
8/4/75	Sunny, Hot Humid following weekend of same	C	OCP, V.O., H.M.	6.9 @ 9:15am	27.0	-	-	-	-	1-2
			Enteric Virus Run	7.2 @ 9:35am	27.0	1.5*	1.4*	Total <2	-	1-2
				7.3 @ 11:30am	26.4	-	-			
		D	OCP, V.O., H.M.	7.6 @ 10:20am	27.5	1.9	1.6	-	-	4
		F	OCP, V.O., H.M.	7.2 @ 11:30am	28.5	0.5	0.2	-	-	4
		E	OCP, V.O., H.M.	7.1 @ 12:50pm	27.0	0.7	0.6	-	-	4
8/5/75	Sunny, Hot Humid following evening of thunderstorms	A	OCP, V.O., H.M.	7.5 @ 8:25am	25.5	-	-	-	-	1
			Enteric Virus Run	7.6 @ 9:06am	25.0	-	-	Total 23,000 (7000-70,000) Fecal 1300 (300-3100)	-	1
				7.3 @ 11:03am	26.5	-	-			
		B	OCD, V.O., H.M.	7.6 @ 12:55pm	28.0	<0.1	0	-	-	1
8/6/75	Cloudy, Chance of Showers 75-80°	B	Enteric Virus Run	7.5 @ 8:25am	26.0	<0.1	0	Total 7900 (2500-19,000) Fecal 1300 (300-3100)	-	1
				7.5 @ 10:00am	26.0	-	-			
8/7/75	Partly Cloudy 70-75°	D	Enteric Virus Run	7.6 @ 9:06am	26.0	1.7	1.5	Total <2	0	4
				7.6 @ 10:16am	26.5	1.8	1.7			
8/11/75	Partly Cloudy 80-85°	B	Enteric Virus Run	7.6 @ 8:39am	29.0	<0.1	0	Total 2300 (700-7000) Fecal 500 (<50-1300)	-	1
				7.5 @ 10:44am	29.0	<0.1	0			
		C	All Pesticides V.O.	7.0 @ 12:50pm	27.0	-	-	-	-	2
		D	All Pesticides V.O.	7.3 @ 1:07pm	27.5	1.7	1.5	-	-	4
		F	OCP, V.O.	7.4 @ 1:54pm	28.5	0.7	0.7	-	-	4
		E	OCP, V.O.	7.4 @ 3:16pm	28.5	0.7	0.5	-	-	4
8/12/75	Partly Sunny Hot 85-90° following evening of heavy thunderstorms	A	All Pesticides V.O.	7.3 @ 9:04am	21.0	-	-	-	-	1-2
			Enteric Virus Run	7.5 @ 9:50am	22.0	-	-	Total 7900 (2500-19000) Fecal 4900 (1700-13000)	-	1-2
				7.2 @ 11:58am	23.5	-	-			

* Chlorine is present due to cleaning of traveling screen chamber for slime removal.

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
8/12/75		B	All Pesticides	7.4 @ 2:10pm	26.5	<0.1	0	-	-	1-2 thick surface scum on stream
8/18/75	Sunny 80-85°	D	OCP, V.O.	7.6 @ 8:49am	28.0	2.4	2.2	-	-	4
			Enteric Virus Run	7.6 @ 9:30am 7.1 @ 11:40am	28.0 26.9	1.7 -	1.5 -	Total <2	0	4
		C	OCP, V.O.	7.5 @ 10:10am	27.0	-	-	-	-	2
		F	OCP, V.O.	7.2 @ 11:05	29.0	0.4	0.4	-	-	4
		E	OCP, V.O.	7.2 @ 12:30	27.0	1.0	0.8	-	-	4
8/19/75	Sunny 80-85°	A	OCP, V.O.	7.4 @ 8:50am	23.0	-	-	-	-	1
			Enteric Virus Run	7.5 @ 9:50am 7.4 @ 11:30am	23.5 25.0	- -	- -	Total 7900 (2500-19000) Fecal 200 (<50-700)	-	1
8/20/75	-	B	Enteric Virus Run	7.4 @ 8:50am 7.3 @ 10:35am	25.0 25.0	<0.1 <0.1	0 0	Total 4900 (1700-13000) Fecal 400 (<50-1100)	-	1
8/21/75	-	C	Enteric Virus Run	6.9 @ 8:58am 7.0 @ 10:35am	27.0 27.0	- -	- -	Total 21 (7-63) Fecal 13 (3-31)	-	1-2
8/25/75	Sunny, Hot Humid following evening of heavy thunderstorms	A	Enteric Virus Run	7.7 @ 9:18am 7.3 @ 11:30am	23.0 27.1	- -	- -	Total 140,000 (37000-340,000) Fecal 17,000 (4300-49,000)	-	3
		C	All Pesticides V.O.	7.1 @ 1:35pm	27.5	-	-	-	-	2 - black particles in water
		D	All Pesticides V.O.	7.2 @ 2:05pm	27.8	1.8	1.7	-	-	4
		F	OCP, V.O.	7.4 @ 2:50pm	29.0	0.8	0.7	-	-	4
		E	OCP, V.O.	7.2 @ 3:55pm	27.2	1.4	1.3	-	-	4

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l		Bacteria		Observations
						Total	Free	MPN	TPC	
8/26/75	Sunny, Hot Humid	B	All Pesticides V.O.	7.2 @ 8:28am	24.8	<0.1	0	-	-	1
			Enteric Virus Run	7.2 @ 9:05am 7.2 @ 10:58am	25.2 26.9	<0.1 <0.1	0 0	Total 4900 (1700-13000) Fecal 700 (100-1700)	-	1
		A	All Pesticides V.O.	7.2 @ 1:35pm	26.4	-	-	-	-	1
8/27/75	Sunny 85-90°	C	Enteric Virus Run	7.1 @ 9:21am 7.0 @ 11:30am	27.5 27.5	- -	- -	Total 33 (11-93) Fecal <2	- -	1-2 1-2
8/28/75	Sunny 80-85°	B	Adeno Seed Run	7.5 @ 8:59am 7.4 @ 10:10am	24.0 24.0	<0.1 <0.1	0 0	-	-	1
			Enteric & Reo Seed Run	7.3 @ 11:05am 7.4 @ 12:10pm	25.0 25.6	<0.1 <0.1	0 0	-	-	1
9/1/75	Rain 70-75° following weekend of heavy rains	C	OCP, V.O.	7.0 @ 9:-5am	25.6	-	-	-	-	2
		D	OCP, V.O.	7.2 @ 10:15am	25.4	2.6	2.1	-	-	4
		E	OCP, V.O.	7.2 @ 11:20am	25.4	1.8	1.8	-	-	4
		F	OCP, V.O.	7.1 @ 1:00pm	27.8	1.5	1.0	-	-	4
9/2/75	Cloudy 80-85°	A	OCP, V.O.	7.4 @ 12:35pm	22.0	-	-	-	-	2
		B	OCP, V.O.	7.2 @ 2:05pm	22.0	-*	-*	-	-	3
9/9/75	Partly Sunny 70-75°	B	All Pesticides V.O., H.M.	7.4 @ 9:00am	22.0	<0.1	0	-	-	1
		A	All Pesticides V.O., H.M.	7.6 @ 10:00am	20.0	-	-	-	-	1
		D	All Pesticidus V.O., H.M.	7.2 @ 11:30am	24.0	2.9	2.7	-	-	4
		C	All Pesticides V.O., H.M.	7.3 @ 12:00N	23.4	-	-	-	-	2-3 more color than usual
		F	OCP, V.O., H.M.	7.2 @ 12:55pm	26.0	2.1	1.9	-	-	4, water very effervescent
		E	OCP, V.O., H.M.	7.2 @ 2:30pm	24.0	1.0	0.5	-	-	4

* Water too turbid to test.

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
9/10/75	Cloudy 60-65°	B	OCP, V.O., H.M.	7.5 @ 8:00am	19.2	<0.1	0	-	-	1
		A	OCP, V.O., H.M.	7.4 @ 9:10am	15.0	-	-	-	-	1
		C	OCP, V.O., H.M.	6.9 @ 10:18am	22.6	-	-	-	-	1, more color than usual
		D	OCP, V.O., H.M.	7.2 @ 10:35am	23.2	2.1	1.7	-	-	4
		F	OCP, V.O., H.M.	7.2 @ 11:20am	25.4	1.6	1.4	-	-	4
		E	OCP, V.O., H.M.	7.3 @ 1:00pm	24.4	0.5	0.4	-	-	4
9/11/75	Partly Cloudy 75-80° Showers in Evening	A	OCP, V.O., H.M.	6.8 @ 8:40am	18.0	-	-	-	-	-
		B	OCP, V.O., H.M.	6.8 @ 9:20am	20.0	-	-	-	-	-
		C	Bi-hourly sampling 12pm - 12pm See Table							
		D	Bi-hourly sampling 12pm - 12pm See Table							
		E	Bi-hourly sampling 12pm - 12pm See Table							
		D	Bi-hourly sampling 12pm - 12pm See Table							
9/12/75	Cloudy 75-80° Showers	A	OCP, V.O., H.M.	7.3 @ 8:00am	21.0	-	-	-	-	1
		B	OCP, V.O., H.M.	7.1 @ 8:45am	22.0	-	-	-	-	1
		F	OCP, V.O., H.M.	7.6 @ 1:25pm	26.0	1.5	1.0	-	-	4,effervescent
		E	OCP, V.O., H.M.	7.5 @ 2:45pm	24.2	1.1	0.8	-	-	4
		C	OCP, V.O., H.M.	6.9 @ 4:45pm	22.6	-	-	-	-	1-2
		D	OCP, V.O., H.M.	7.2 @ 5:25pm	22.6	2.9	2.3	-	-	4
9/13/75	Sunny, Cool Windy 60-65°	B	OCP, V.O., H.M.	7.3 @ 10:50am	18.0	<0.1	0	-	-	1-2
		A	OCP, V.O., H.M.	7.7 @ 11:35am	15.0	-	-	-	-	1
		C	OCP, V.O., H.M.	7.2 @ 12:45pm	22.2	-	-	-	-	1-2
		D	OCP, V.O., H.M.	7.3 @ 1:00pm	22.4	2.2	1.3	-	-	4
		F	OCP, V.O., H.M.	7.3 @ 1:45pm	25.3	1.8	1.2	-	-	4,effervescent
		E	OCP, V.O., H.M.	7.4 @ 2:53pm	23.7	0.9	0.5	-	-	4
9/14/75	Sunny, Cool 65-70°	B	OCP, V.O., H.M.	7.4 @ 10:55am	17.0	0	0	-	-	1
		A	OCP, V.O., H.M.	7.3 @ 11:50am	14.0	-	-	-	-	1,approaching clear and colorless

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Free	Bacteria MPN	TPC	Observations
9/14/75	Sunny, Cool 65-70°	C	OCP, V.O., H.M.	6.9 @ 12:55pm	22.0	-	-	-	-	1
		D	OCP, V.O., H.M.	7.1 @ 1:15pm	21.0	2.2	1.8	-	-	4
		F	OCP, V.O., H.M.	7.2 @ 1:40pm	25.0	1.3	1.0	-	-	4,effervescent
		E	OCP, V.O., H.M.	7.3 @ 3:00pm	24.0	1.5	0.5	-	-	4
9/15/75	Partly Cloudy 65-70°	B	OCP, V.O., H.M.	7.5 @ 9:45am	15.0	0	0	-	-	1,approaching clear and colorless
		A	OCP, V.O., H.M.	7.5 @ 10:30am	12.0	-	-	-	-	1, approaching clear and colorless
		C	OCP, V.O., H.M.	6.7 @ 11:30am	22.0	-	-	-	-	1-2
		D	OCP, V.O., H.M.	7.2 @ 11:50am	22.0	2.9	2.2	-	-	4
		F	OCP, V.O., H.M.	7.0 @ 12:15pm	25.0	3.0	2.5	-	-	4, very effervescent
		E	OCP, V.O., H.M.	7.3 @ 2:00pm	23.0	1.0	0.7	-	-	4
9/16/75	Partly Sunny 75-80°	B	OCP, V.O.	7.5 @ 8:55am	15.5	<0.1	0	-	-	1,approaching clear and colorless
		A	OCP, V.O.	7.3 @ 10:00am	14.0	-	-	-	-	1,approaching clear and colorless
		C	OCP, V.O.	6.8 @ 11:10am	21.5	-	-	-	-	1-2, more color than usual
		D	OCP, V.O.	7.0 @ 11:30am	22.0	2.8	2.2	-	-	4
		F	OCP, V.O.	7.0 @ 12:35pm	24.0	2.0	1.5	-	-	4
		E	OCP, V.O.	7.1 @ 3:10pm	22.5	2.0	1.5	-	-	4
9/22/75	Cloudy 70-75°	C	All Pesticides V.O.	7.1 @ 9:35am	21.5	-	-	-	-	1
		D	All Pesticides V.O.	7.2 @ 10:05am	21.5	3.5	3.0	-	-	4
		F	OCP, V.O.	7.3 @ 12:45pm	24.0	1.8	1.6	-	-	4
		E	OCP, V.O.	7.7 @ 3:20pm	22.0	2.1	1.9	-	-	4

TABLE B-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
9/23/75	Heavy Rains 55-60°	B	All Pesticides V.O.	7.2 @ 12:05pm	16.5	-*	-*	-	-	3
		A	All Pesticides V.O.	7.3 @ 1:17pm	16.5	-	-	-	-	3
9/29/75	Sunny 70-75°	E	OCP, V.O.	7.3 @ 11:10pm	20.0	3.0	2.5	-	-	4
		F	OCP, V.O.	7.5 @ 1:50pm	21.5	2.0	1.6	-	-	4
		C	OCP, V.O.	7.0 @ 3:05pm	18.0	-	-	-	-	3
		D	OCP, V.O.	7.0 @ 3:45pm	19.0	2.2	1.7	-	-	4
9/30/75	Sunny 70-75°	A	OCP, V.O.	7.5 @ 8:30am	13.5	-	-	-	-	1
		B	OCP, V.O.	7.4 @ 9:20am	15.0	-	-	-	-	1-2
10/6/75	Sunny 70-75°	E	OCP, V.O., H.M.	7.8 @ 10:15am	19.0	1.4	1.2	-	-	4
		F	OCP, V.O., H.M.	7.6 @ 12:00N	22.5	2.3	1.7	-	-	4
		C	All Pesticides V.O., H.M.	6.7 @ 1:00pm	18.5	-	-	-	-	1
		D	All Pesticides V.O., H.M.	7.1 @ 1:38pm	19.5	2.3	2.1	-	-	4
10/7/75	Partly Sunny 70-75°	A	All Pesticides V.O., H.M.	7.6 @ 8:25am	13.0	-	-	-	-	1,approaching clear and colorless
		B	All Pesticides V.O., H.M.	7.4 @ 11:45am	15.0	<0.1	0	-	-	1
10/8/75	Partly Sunny 70-75°	E	V.O., H.M.	7.6 @ 10:06am	18.5	1.4	1.1	-	-	4
		F	V.O., H.M.	7.4 @ 1:20pm	21.0	1.6	1.3	-	-	4
11/10/75	Partly Sunny in afternoon, Showers in morning	E	OCP, V.O., H.M.	7.1 @ 9:55am	17.5	3.1	2.6	-	-	4
		F	OCP, V.O., H.M.	7.1 @ 1:20pm	19.0	2.5	2.2	-	-	4
		C	All Pesticides V.O., H.M.	6.9 @ 2:25pm	16.0	-	-	-	-	1
		D	All Pesticides V.O., H.M.	7.1 @ 2:50pm	16.5	2.9	2.5	-	-	4
11/11/75		B	All Pesticides V.O., H.M.	7.5 @ 2:35pm	15.5	0	0	-	-	1

* Water too turbid to test.

TABLE B-1 (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
11/11/75		A	All Pesticides V.O., H.M.	8.1 @ 3:45pm	14.0	-	-	-	-	1,approaching clear and colorless
12/8/75	Cold 30-35° Snow/Rain	E	OCP, V.O., H.M.	7.0 @ 9:30am	9.5	2.3	2.3	-	-	4
		F	OCP, V.O., H.M.	6.8 @ 10:40am	11.0	2.1	1.6	-	-	4,effervescent
		C	OCP, V.O., H.M.	6.2 @ 2:00pm	8.0	-	-	-	-	1
		D	OCP, V.O., H.M.	6.9 @ 2:40pm	9.0	2.6	2.1	-	-	4
12/9/75	-	B	OCP, V.O., H.M.	6.6 @ 12:45pm	4.0	-*	-*	-	-	2
		A	OCP, V.O., H.M.	6.9 @ 1:05pm	4.0	-	-	-	-	1
1/12/76	Sunny, Cold 30-35°	E	OCP, V.O., H.M.	7.6 @ 10:08am	4.5	2.1	2.0	-	-	4,In V.O. sampling area slight odor of moth balls
		F	OCP, V.O., H.M.	7.7 @ 11:18am	6.0	2.5	2.2	-	-	4, very effervescent
		C	OCP, V.O., H.M.	7.2 @ 2:45pm	3.0	-	-	-	-	1,2,Caulking matl odor in carbon bldg due to construction
		D	OCP, V.O., H.M.	7.6 @ 3:23pm	4.0	2.9	2.9	-	-	4
1/13/76	Partly Cloudy 40-45°	B	OCP, V.O., H.M.	7.7 @ 12:20pm	2.0	<0.1	0	-	-	1
		**A	OCP, V.O., H.M.	7.3 @ 1:50pm	2.0	-	-	-	-	1,approaching clear and colorless
1/27/76	Heavy Rain 50-55°	A	H.M.	6.3 @ 10:40am	2.5	-	-	-	-	3
2/17/76	Partly Sunny 75-80°	E	OCP, V.O., H.M.	7.5 @ 10:45am	9.0	2.1	1.4	-	-	4,In V.O. sampling area mothball odor
		F	OCP, V.O., H.M.	6.9 @ 1:40pm	11.0	2.1	1.3	-	-	4

* Water too turbid to test.

** Sampling repeated 1/27/76 due to contamination on 1/13/76.

TABLE B-4. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l Total	Chlorine Residual, mg/l Free	Bacteria MPN	Bacteria TPC	Observations
2/17/76	Partly Sunny 75-80°	*C	OCP, V.O., H.M.	6.8 @ 2:40pm	6.0	-	-	-	-	3
		D	OCP, V.O., H.M.	7.3 @ 3:05pm	9.5	2.2	1.9	-	-	4
2/18/76	Partly Cloudy 70-75° Windy	B	OCP, V.O., H.M.	7.6 @ 10:10am	11.0	<0.1	0	-	-	1-2
		A	OCP, V.O., H.M.	7.7 @ 11:45am	11.0	-	-	-	-	1
3/8/76	Cloudy Cool 50°	E	OCP, V.O., H.M.	7.6 @ 10:20am	10.5	2.0	1.7	-	-	4, In V.O. sampling area slight odor of moth balls
		F	OCP, V.O., H.M.	7.2 @ 12:35pm	11.0	1.9	1.5	-	-	4
		C	OCP, V.O., H.M.	6.9 @ 3:20pm	9.5	-	-	-	-	1
		D	OCP, V.O., H.M.	7.7 @ 3:55pm	10.5	2.0	1.8	-	-	4
3/9/76	Cold, Snow	B	OCP, V.O., H.M.	7.7 @ 11:10pm	6.0	<0.1	<0.1	-	-	1
		A	OCP, V.O., H.M.	7.8 @ 12:00M	3.0	-	-	-	-	1
4/12/76	Sunny, Cool 45-50°	E	OCP, V.O., H.M.	7.5 @ 10:50am	11.0	1.5	0.7	-	-	4
		F	OCP, V.O., H.M.	8.0 @ 1:45pm	13.0	1.2	0.7	-	-	4
		C	OCP, V.O., H.M.	7.6 @ 2:45pm	11.0	-	-	-	-	2-3
		D	OCP, V.O., H.M.	7.2 @ 3:20pm	15.5	1.3	1.0	-	-	4
4/13/76	Sunny 60-65°	A	OCP, V.O., H.M.	8.3 @ 3:00pm	13.5	-	-	-	-	1,approaching clear and colorless
		B	OCP, V.O., H.M.	7.6 @ 2:00pm	11.0	0	0	-	-	1,approaching clear and colorless
5/10/76	Sunny, Warm 75-80° Very Dry Period	E	OCP, V.O., H.M.	7.4 @ 11:00am	19.5	1.8	1.1	-	-	4
		F	OCP, V.O., H.M.	7.5 @ 2:10pm	19.5	1.9	1.7	-	-	4
		C	OCP, V.O., H.M.	7.1 @ 3:05pm	17.5	-	-	-	-	1
		B	OCP, V.O., H.M.	7.4 @ 3:30pm	18.5	2.6	2.4	-	-	4

* Traveling screen chamber out of service for screen installation.

TABLE II-1. (continued)

Sampling Date	Weather Conditions	Sampling Site	Analyses Performed	pH and Time	Temperature °C	Chlorine Residual, mg/l		Bacteria		Observations
						Total	Free	MPN	TPC	
5/11/76	Cloudy 65-70°F	B	OCP, V.O., H.M.	7.3 @ 8:30am	17.0	0	0	-	-	1, approaching clear and colorless
		A	OCP, V.O., H.M.	7.4 @ 9:20am	15.5	-	-	-	-	1, approaching clear and colorless

Appendix C

Field and Laboratory Data Pertaining to Occoquan-II Environmental Sampling Program for Viruses

TABLE C-1. LABORATORY AND FIELD DATA ASSOCIATED WITH THE SAMPLING AND ANALYSES BY THE CARBORUNDUM COMPANY OF ALL WATER SAMPLES FOR ENTEROVIRUS DURING OCCOQUAN-II, JUNE, 1976 - MARCH, 1977.

Sampling Date	Weather Conditions	Site	Time	Duration, hrs	Volume Sampled, gal	UNH Lab No.	Assay Date	Assay Results	pH	Time	Temp °C	Cl_2 Residual, mg/l Total	Cl_2 Residual, mg/l Free	Bacteria MPN/100 ml	Bacteria TPC/ml	^{a}Observations
6/17/76	Pt. cloudy, hot, humid following evening of thunderstorms	C	12:32 pm	2.5	112	^{b}664-C ^{b}665-A	7/9 7/9	$^{c}0$ $^{c}0$	7.1 7.1	12:15 pm 3:15 pm	22.0 22.0	- -	- -	Total 70 (43-490) Fecal <2	1000	1
6/18/76	Pt. cloudy hot, humid	D	12:25 pm	2.0	107.5	666-A	7/9	$^{c}0$	7.3 7.3	11:50 am 2:20 pm	23.5 25.5	3.0 2.75	2.75 2.5	Total <2	0	4
6/21/76	Heavy Rain 75-80° following week end of heavy rains	E	11:15 am	2.0	108	667-A	7/9	$^{c}0$	7.3 7.2	10:50 am 1:20 pm	22.5 22.0	2.4 2.3	2.2 2.0	Total <2	0	4
6/22/76	Sunny 80-85°F	F	11:20 am	2.0	101	668-A	7/9	$^{c}0$	7.3 7.4	11:15 am 1:15 pm	24.5 24.0	2.2 2.4	2.1 2.2	Total <2	0	4
6/23/76	Sunny Hot	C	10:44 am	2.2	105	669-C 670-A	7/9 7/9	$^{c}0$ $^{c}0$	7.2 7.1	10:35 am 1:00 pm	23.0 23.0	- -	- -	Total >0 (23-170) Fecal 7 (1-17)	230	1
6/24/76	Sunny, Hot 80-85°F	D	10:23 am	2.0	109.5	672-A	7/9	$^{c}0$	7.5 7.3	10:00 am 12:55 pm	23.5 24.5	3.0 2.3	2.8 2.2	Total <2	0	4
6/25/76	Variable Cloudiness 80-85°F	E	9:48 am	2.1	112	671-A	7/9	$^{c}0$	7.5 7.5	9:45 am 12:35 pm	23.0 22.5	2.3 2.4	2.1 2.1	Total <2	0	4
6/28/76	Sunny, Hot	F	11:02 am	2.0	108	676-A	7/22	$^{c}0$	7.4 7.4	10:45 am 1:10 pm	26.5 26.0	2.3 2.7	1.6 2.5	Total <2	0	4

(continued)

TABLE C-1. (continued)

Sampling Date	Weather Conditions	Site	Time	Dura-tion, hrs	Volume Sampled (gal)	UNH Lab No.	Assay Date	Assay Results	pH	Time	Temp °C	Cl_2 Residual, mg/l		Bacteria		[a]Observa-tions
												Total	Free	MPN/100 ml	TPC/ml	
6/29/76	Sunny, Hot	C	10:52 am	1.7	105	677-A	7/22	$^{c}0$	7.1	10:25 am	24.0	-	-	Total 13 (3-31)	300	1
									7.0	12:50 pm	24.0	-	-	Fecal 8 (1-19)		
6/30/76	Pt. Sunny 80-85°F	D	10:18 am	1.9	119	678-A	7/22	$^{c}0$	7.4	10:00 am	24.5	3.0	2.8	Total <2	0	4
									7.4	12:30 pm	25.0	2.7	2.7			
7/1/76	Pt. Sunny 80-85° F following evening thunderstorms	E	10:04 am	2.0	112	679-A	7/22	$^{c}0$	7.2	9:40 am	25.0	2.8	2.5	Total <2	0	4
									7.5	12:00 pm	23.5	2.8	2.6			
7/2/76	Sunny, 80-85° F	F	10:28 am	1.7	104	680-A	7/22	$^{c}0$	7.4	10:05 am	26.0	1.6	1.4	Total <2	0	4
									7.4	12:15 pm	26.0	1.4	1.1			
7/6/76	Sunny, 80-85° F following Week-end of light rain showers	C	11:30 am	1.7	103	681-C	7/22	$^{c}0$	7.2	11:25 am	24.5	-	-	Total 130 (35-300)	180	1
						682-A	7/22	$^{c}0$	7.2	1:15 pm	24.5	-	-	Fecal 2 (<0.5-7)		
7/7/76	Cloudy 75-80° fol-lowing heavy showers in am	D	10:17 am	1.7	103	683-A	7/22	$^{c}0$	7.6	9:55 am	24.0	2.7	2.5	Total <2	3	4
									7.4	11:55 am	24.5	3.1	2.6			
7/8/76	Pt. Sunny following eve-ning of heavy thunderstorms	E	9:45 am	2.0	106	684-A	7/22	$^{c}0$	7.4	9:20 am	24.0	2.3	2.1	Total <2	0	4
									7.4	11:55 am	24.5	2.3	2.2			
7/9/76	Hazy 85-90°F	F	10:17 am	1.6	104	685-A	7/22	$^{c}0$	7.7	10:05 am	26.0	1.3	1.1	Total <2	0	4
									7.6	11:55 am	26.5	1.2	1.0			

(continued)

TABLE C-1.(continued)

Sampling Date	Weather Conditions	Site	Time	Duration, hrs	Volume Sampled (gal)	UNH Lab No.	Assay Date	Assay Results	pH	Time	Temp °C	Cl_2 Residual, mg/l Total	Cl_2 Residual, mg/l Free	Bacteria MPN/100 ml	Bacteria TPC/ml	^{a}Observations
7/12/76	Sunny, following week-of numerous heavy thunderstorms	C	10:13 am	1.8	103	715-C	7/27	$^{c}0$	7.2	10:00 am	25.0	-	-	Total 33 (11-93) Fecal <2	450	1
						716-A	7/27	$^{c}0$								
7/13/76^{d}	Sunny, Breezy	D	10:00 am	1.8	106	717-A	7/27	$^{c}0$	7.8 7.8	9:35 am 11:50 am	24.5 24.5	2.2 2.0	2.1 2.0	Total <2	7	4
7/14/76^{d}	Sunny,	E	9:45 am	2.0	104	718-A	7/27	$^{c}0$	7.8 7.7	9:30 am 11:50 am	24.0 24.0	1.6 1.8	1.3 1.2	Total <2	0	4
7/15/76^{d}	Sunny, Hot Humid following early morning thunderstorms	C	11:20 am	2.0	102	719-C	7/27	$^{c}0$	7.4	11:00 am	25.0	-	-	Total 33 (11-93) Fecal 5 (<.5-13)	300	1
						720-A	7/27	$^{c}0$	7.4	1:20 pm	25.5	-	-			
7/16/76^{d}	Sunny 85-90°F humid following thunderstorms	D	9:42 am	1.7	105	721-A	7/27	$^{c}0$	7.2 7.0	9:25 am 11:30 am	25.5 26.0	2.5 2.5	2.2 2.5	Total <2	0	4
7/19/76^{d}	Sunny 85-90°F	F	12:20 pm	1.6	102	722-A	7/27	$^{c}0$	7.2 7.2	12:10 pm 2:05 pm	27.0 27.0	2.2 2.2	2.0 2.0	Total <2	0	4
7/20/76^{d}	Sunny, 85-90°F	E	9:50 am	1.3	109	723-A	7/27	$^{c}0$	7.3 7.4	9:30 am 11:10 am	25.0 25.0	2.4 2.5	2.0 2.2	Total <2	3	4
7/21/76^{d}	Pt. Sunny, Hot, Humid	F	10:25 am	1.5	100	724-A	8/6	$^{c}0$	7.2 7.2	10:12 am 12:11 pm	27.0 27.0	1.7 1.6	1.5 1.2	Total <2	0	4
7/22/76^{d}	Cloudy, humid, light rain in am	C	10:50 am	1.9	101	725-C	8/6	$^{c}0$	7.0	10:40 am	25.5	-	-	Total 11 (2-25) Fecal 2 (<0.5-7)	e	1
						726-A	8/6	$^{c}0$	7.1	12:50 pm	25.5	-	-			

(continued)

TABLE C-1 (continued)

Sampling Date	Weather Conditions	Site	Time	Duration, hrs	Volume Sampled (gal)	UNH Lab No.	Assay Date	Assay Results	pH	Time	Temp °C	Cl_2 Residual, mg/l Total	Free	Bacteria MPN/100 ml	TPC/ml	Observations
7/23/76[d]	Cloudy, 75-80°F, light	D	9:50 am	1.9	105	727-A	8/6	$^{c}0$	7.4 7.6	9:40 am 11:45 am	25.0 25.5	2.6 2.5	2.3 2.3	Total <2	3	4
7/26/76	Sunny, 80-85°F	E	10:14 am	1.7	100	728-A	8/6	$^{c}0$	7.4 7.6	9:40 am 11:45 am	25.0 25.5	2.6 2.5	2.3 2.3	Total <2	0	4
7/27/76[d]	Sunny, 85-90°F	F	10:15 am	1.7	102	731-A	8/6	$^{c}0$	7.3 7.3	10:00 am 12:05 pm	27.0 27.5	2.1 2.0	1.9 1.8	Total <2	0	4
7/28/76[d]	Sunny, Hot Humid, 90-95°	C	10:27 am	2.3	100	732-C	8/6	$^{c}0$	7.0	10:25 am	26.0	-	-	Total >9 (25-190) Fecal <2	630	1 musty odor
						733-A	8/6	$^{c}0$	7.1	1:50 pm	26.5	-	-			
7/29/76	Pt. Sunny, Hot, Humid following evening thunderstorms	D	9:51 am	1.9	100	734-A	8/6	$^{c}0$	7.4 7.4	9:30 am 11:50 am	26.0 26.5	3.0 2.6	2.4 2.3	Total <2	7	4 musty odor
7/30/76	Pt. Sunny 80-85°F following evening thunderstorms	E	9:40 am	1.8	103	725-A	8/6	$^{c}0$	7.4 7.4	9:25 am 11:30 am	26.0 26.0	1.9 2.2	1.8 1.8	Total <2	0	4 musty odor
8/2/76	Pt. Sunny, 75-80°F	F	10:25 am	1.7	109	736-A	8/6	$^{c}0$	7.2 7.3	10:10 am 12:10 pm	27.0 27.5	1.7 1.7	1.5 1.5	Total <2	0	4
8/3/76	Sunny 75-80°F	C	10:14 am	1.9	100	737-C	8/13	$^{c}0$	7.0	9:55 am	25.5	-	-	Total 110 (31-250) Fecal 2 (<0.5-7)	67	1 musty odor
						738-A	8/13	$^{c}0$	7.0	12:10 pm	26.5	-	-			
8/4/76	Sunny, 75-80°	D	9:44 am	1.7	112	739-A	8/13	$^{c}0$	7.2 7.3	9:25 am 11:32 am	25.5 26.0	2.4 2.1	2.2 2.0	Total <2	0	4

(continued)

TABLE C-1. (continued)

Sampling Date	Weather Conditions	Site	Time	Duration, hrs	Volume Sampled (gal)	UNH Lab No.	Assay Date	Assay Results	pH	Time	Temp °C	Cl_2 Residual, mg/l Total	Cl_2 Residual, mg/l Free	Bacteria MPN/100 ml	Bacteria TPC/ml	Observations
8/5/76	Sunny, 80-85°F	E	9:37 am	1.6	101	740-A	8/13	^{c}0	7.4 7.5	9:10 am 11:20 am	25.0 25.5	2.1 2.3	1.9 1.9	Total <2	13	4
8/6/76	Sunny, 85-90°F	F	9:50 am	1.6	120	740-A	8/13	^{c}0	7.3 7.3	9:35 am 11:30 am	27.5 27.5	1.8 1.9	1.5 1.6	Total <2	0	4
8/9/76	Rain, 75-80° following week-end of heavy rains	C	10:50 am	1.8	100	742-C	8/13	^{c}0	7.0	10:30 am	25.0	-	-	Total 220 (57-700) Fecal 8 (1-19)	600	1-2 H_2S odor in area. Susp. matter evident in H_2O
						743-A	8/13	^{c}0	6.9	12:45 pm	-	-	-			
8/10/76	Sunny, 80-85°F	D	10:17 am	1.7	103	757-A	8/18	^{c}0	7.1 7.8	9:35 am 12:10 pm	25.5 25.4	2.4 3.0	2.2 2.7	Total <3	7	4
8/11/76	Sunny, 85-90°F	E	9:44 am	1.9	103	758-A	8/18	^{c}0	7.4 7.6	9:35 am 11:52 am	24.5 24.6	1.6 2.2	1.4 1.8	Total <3	0	4
8/12/76	Sunny, Hot 85-90°F	F	10:27 am	1.8	117	759	8/18	^{c}0	7.4 7.4	10:10 am 12:30 pm	27.0 27.4	2.1 2.6	1.7 2.2	Total <3	0	4
8/13/76	Sunny, Hot Humid 95°F	C	10:01 am	1.8	100	760-C	8/18	^{c}0	7.0	9:15 am	26.0	-	-	Total 130 (35-300) Fecal <2	125	1 color > than usual
						761-A	8/18	^{c}0	6.9	12:15 pm	26.2	-	-			
8/16/76	Pt. Sunny 75-80° following week-end of thunderstorms	D	10:54 am	1.5	101	762-A	8/18	^{c}0	7.6 7.3	9:15 am 11:30 am	25.0 25.0	3.1 3.0	2.6 2.6	Total <3	0	4
8/17/76	Sunny, 75-80°F	E	9:36 am	1.9	105	792-A	9/14	^{c}0	7.4 7.3	9:15 am 11:25 am	24.5 25.0	2.2 2.1	2.0 1.9	Total <3	0	4

(continued)

TABLE C-1 (continued)

Sampling Date	Weather Conditions	Site	Time	Duration, hrs	Volume Sampled (gal)	UNH Lab No.	Assay Date	Assay Results	pH	Time	Temp °C	Cl_2 Residual, mg/l		Bacteria		Observations
												Total	Free	MPN/100 ml	TPC/ml	
8/18/76	Sunny, 80-85°F	F	10:28 am	2.0	101	793-A	9/14	^{c}0	7.4 7.4	10:15 am 12:10 pm	27.0 27.0	2.5 2.2	2.3 2.0	Total <3	0	4
8/19/76	Sunny, 80-85°F	C	10:22 am	1.9	100	794-C	9/14	^{c}0	7.0	9:55 am	25.0	-	-	Total 130 (35-300)	300	1 color > usual, > turb. at 12:10 pm
						795-A	9/14	^{c}0	7.0	12:10 pm	25.5	-	-	Fecal 13		
8/20/76	Sunny, 80-85°F	D	11:54 am	1.8	100	796-A	9/14	^{c}0	7.2 7.2	11:25 am 1:35 pm	24.5 25.0	2.9 2.9	2.5 2.6	Total <3	0	4
8/23/76	Sunny, 80-85°F	E	9:20 am	1.6	100	797-A	9/14	^{c}0	7.5 7.4	9:00 am 11:10 am	25.0 25.5	2.5 2.7	2.4 2.6	Total <3	0	4
8/24/76	Hazy, Humid 85-90°, Air Poll. Alert	F	10:10 am	1.9	100	798-A	9/14	^{c}0	7.4 7.4	9:56 am 12:10 pm	27.5 28.0	2.6 2.4	2.3 2.2	Total <3	0	4
8/25/76	Hazy, Hot Humid, Air Poll. Alert	C	10:08 am	1.9	101	799-C	9/14	^{c}0	7.0	9:52 am	25.5	-	-	Total 17 (5-46)	360	1 musty odor
						800-A	9/14	^{c}0	7.0	12:03 pm	26.0	-	-	Fecal <2		
8/27/76	Cloudy, light rain	E	10:35 am	1.8	100	801-A	9/14	^{c}0	7.4 7.2	10:20 am 12:10 pm	26.0 25.5	2.3 2.6	2.1 2.3	<3	0	4
8/30/76	Sunny, cool breezy, 75°	F	10:24 am	1.3	101	802-A	9/14	^{c}0	7.1 7.1	10:07 am 11:54 am	26.0 27.0	2.2 2.3	1.9 2.1	<3	0	4
8/31/76	Sunny, cool 70-75°F	C	10:12 am	1.9	101	807-C	9/20	^{c}0	7.1	9:56 am	24.5	-	-	Total 27 (9-80)	160	1 suspended matter present
						808-A	9/20	^{c}0	6.8	12:04 pm	25.0	-	-	Fecal <2		
9/1/76	Sunny, 75-80°	D	9:37 am	1.8	104	809-A	9/20	^{c}0	7.4 7.3	9:25 am 11:15 am	24.0 25.5	3.8 3.5	3.3 3.2	Total <3	0	4

(continued)

TABLE C-1 (continued)

Sampling Date	Weather Conditions	Site	Time	Duration, hrs	Volume Sampled (gal)	UNH Lab No.	Assay Date	Assay Results	pH	Time	Temp °C	Cl_2 Residual, mg/l Total	Cl_2 Residual, mg/l Free	Bacteria MPN/100 ml	Bacteria TPC/ml	Observations
9/2/76	Rain, 70-75°F	E	9:13 am	1.9	102	810-A	9/20	F_1	7.2 7.3	9:57 am 10:58 am	24.5 24.5	2.4 2.4	2.3 2.1	Total <3	0	4
9/3/76	Cloudy, 70°F	F	9:53 am	1.7	102	811-A	9/20	C_0	7.6 7.6	9:34 am 11:29 am	25.5 26.0	1.4 1.9	1.2 1.6	Total <3	0	4
9/27/76	Cloudy, Rain	D	11:18 am	1.6	104	822-A	10/22	C_0	7.4 7.6	10:50 am 1:05 pm	22.0 23.0	2.9 3.0	2.4 2.4	Total <3	0	4
9/28/76	Sunny, cool 65-70°F	D	10:05 am	2.0	89	823-A	10/22	C_0	7.6 7.8	9:50 am 12:15 pm	21.5 21.5	2.0 2.0	1.5 1.7	<3	0	4
9/29/76	Cloudy, cool, 60-65°	E	9:20 am	2.3	101	824-A	10/22	C_0	7.4 7.3	9:20 am 11:45 am	21.0 21.0	1.5 1.5	1.2 1.2	<3	0	4
9/30/76	Rain, 55-60°F	F	11:35 am	1.7	104	825-A	10/22	C_0	7.2 7.2	11:15 am 1:10 pm	22.0 22.0	2.2 2.4	1.9 2.1	<3	0	4
10/1/76	Rain, 55-60°F	D	10:18 am	2.1	101	826-A	10/22	C_0	7.4 7.2	10:00 am 12:10 pm	20.0 20.0	3.0 3.0	2.6 2.6	<3	0	4
10/4/76	Pt. Cloudy, 70-75°F	E	12:42 pm	1.8	100	876-A	10/22	C_0	7.6 7.5	12:21 pm 2:30 pm	20.5 21.0	2.5 2.6	2.1 2.4	<3	0	4
10/5/76	Sunny, 70-75°F	F	10:24 am	1.3	101	877-A	sample lost		7.4 7.3	10:00 am 12:00 pm	21.5 21.5	2.3 2.4	2.0 2.1	<3	3	4
10/6/76	Pt. Cloudy 70°F	D	11:22 am	1.3	101	878-A	10/22	C_0	7.4 7.4	10:45 am 12:55 pm	18.5 19.0	2.9 2.9	2.3 2.4	<3	7	4
10/7/76	Pt. Cloudy 70°F	E	9:47 am	1.7	101	879-A	10/22	C_0	7.4 7.2	9:20 am 11:22 am	19.5 20.0	2.5 2.5	2.1 2.3	<3	3	4
10/8/76	Cloudy, light rain	F	10:20 am	1.8	105	880-A	10/22	C_0	7.0 7.0	10:00 am 12:00 pm	21.0 21.5	2.1 2.0	1.9 1.7	<3	0	4

(Continued)

TABLE C-1 (continued)

Sampling Date	Weather Conditions	Site	Time	Duration, hrs	Volume Sampled gal	UNH Lab No.	Assay Date	Assay Results	pH	Time	Temp °C	Cl_2 Residual, mg/l Total	Free	Bacteria MPN/100 ml	TPC/ml	[a]Observations
10/11/76	Sunny, cool following week end of heavy rains and high winds	C	10:43 am	2.8	48	[b]900-C	11/3	[h]1	7.0	10:14 am	16.0	-	-	>24000	$4.0x10^4$	3
						[b]901-A	11/3	[c]0	6.9	1:30 pm	17.0	-	-	(Fecal:5400)		
10/12/76	Sunny, Cool 45-50°F	B	11:31 am	3.0	79	903-C	11/3	[g]1	6.8	11:20 am	15.0	<0.1	0	Total >900 (2500-19000)	2000	1
						902-A	11/3	[c]0	7.0	2:35 pm	15.5	0	0	Fecal 630 (210-1500)		
10/13/76[d]	Sunny, 65-70°F	North-side STP	10:15 am	1.4	2.2	905-C	11/3	[j]	7.2	10:15 am	21.5	0	0	Total $46x10^5$ $(16-120x10^5)$	$14x10^4$	1-2
						904-A	11/3	[j]	7.0	11:42 am	22.5	0	0	Fecal $23x10^5$ $(7-70x10^5)$		
10/14/76[d]	Sunny, Windy, 70-75°F	North-side STP	9:53 am	1.6	50	906-C	11/3	[j]	7.0	9:35 am	17.0	2.3	0.3	Total 3500 (120-10,000)	880	1
						907-A	11/3	[j]	7.2	11:32 am	19.0	2.2	0.2	Fecal 49 (17-130)		
10/15/76	Sunny Windy, 70°F	A	10:02 am	3.3	100	909-C	11/3	[c]0	7.3	10:02 am	13.0	-	-	Total 2800 (900-8500)	2500	1
						908-A	11/3	[g]1	7.3	1:23 pm	17.5	-	-	Fecal 220 (70-670)		
3/14/77	Pt. cloudy 55-60°F	C	10:55 am	2.1	100	1230-A	4/22	[c]0	7.1	10:50 am	8.5	-	-	Total 130	190	1-2
						1231-C	4/22	[c]0	7.2	1:05 pm	9.5	-	-	(35-300) Fecal <2		

(continued)

TABLE C-1. (continued)

Sampling Date	Weather Conditions	Site	Time	Duration, hrs	Volume Sampled gal	UNH Lab. No.	Assay Date	Assay Results	pH	Time	Temp °C	Cl$_2$ Residual, mg/l		Bacteria		^{a}Observations
												Total	Free	MPN/100 ml	TPC/ml	
3/15/77	Sunny, 65-70°F	D	10:02 am	1.5	100	1232-A	4/22	^{c}0	7.2	9:55 am	9.5	2.6	2.3	Total <3	0	4
									7.4	11:50 am	10.5	2.4	2.4			
3/16/77	Sunny, Windy 65-70°F	E	9:02 am	1.6	100	1233-A	4/22	^{c}0	7.5	8:50 am	10.0	2.0	2.0	Total <3	7	4
									7.3	10:35 am	10.0	2.1	2.0			
3/17/77	Sunny, 55-60°F	^{1}H	9:07 am	3.0	75	1234-A	4/22	^{c}0	7.4	9:05 am	13.0	0	0	Total 13 (3-31)	47	4
									7.5	12:00 pm	18.0	0	0	Fecal <3		
3/18/77	Rain, 50-55°F	D	8:41 am	1.7	100	1235-A	4/22	^{c}0	7.1	8:35 am	11.0	2.6	2.3	Total <3	0	4
									7.4	10:25 am	11.0	2.6	2.4			
3/21/77	Pt. Sunny 50-55°F	E	9:02 am	1.5	100	1236/A	4/22	^{c}0	7.4	9:00 am	11.0	2.5	2.1	Total <3	0	4
									7.4	10:35 am	11.0	2.2	2.2			
3/22/77	Heavy Rain 45-50°F	C	8:20 am	2.6	100	1237/A	4/22	^{c}0	6.9	8:26 am	10.0	-	-	Total >9 (25-190)	320	1-2
						1238-C	4/22	^{c}0	7.0	11:10 am	10.5	-	-	Fecal 8 (1-19)		
3/23/77	Sunny, High Winds, 45-50°F	D	8:26 am	1.5	100	1341-A	5/25	0	7.2	8:10 am	9.0	2.8	2.5	Total <3	0	4
									7.3	9:55 am	10.0	2.7	2.6			
3/24/77	Sunny, High Winds, 50-55°F	^{1}H	8:50 am	3.2	75	1342-A	5/25	^{c}0	7.3	8:55 am	10.5	0	0	Total 11 (2-25)	150	4
									7.3	12:03 pm	13.5	0	0	Fecal 2 (<0.5-7)		

(continued)

TABLE C-1.(continued)

Sampling Date	Weather Conditions	Site	Time	hrs	Volume Sampled gal	UNH Lab. No.	Assay Date	Assay Results	pH	Time	Temp C	Cl2 Residual, mg/l Total	Cl2 Residual, mg/l Free	Bacteria MPN/100 ml	Bacteria TPC/ml	[a]Observations
3/25/77	Sunny, Cool 50-55°F, Windy	E	8:58 am	1.6	100	1363-A	5/25	[c]0	7.2 7.5	9:00 am 11:45 am	9.5 10.0	1.4 2.0	1.1 1.9	Total <3	0	4

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[a] 1 = Low Turbidity, No Unusual Odor or Color, 2 = Mod. Turb., No Unusual Odor or Color, 3 = High Turb., No Unusual Odor or Color, 4 = Clear Colorless, No Unusual Odor

[b] C: clarifying filter eluate; A: adsorbing filter eluate

[c] Enteroviruses were negative (no plaques) on BGM and PMK cell lines.

[d] Comparative Virus Sampling Between The Carborundum Company and EPA/Cincinnati.

[e] Incubator failure - unreliable results.

[f] One plaque observed on BGM cells. Identified as Polio I. On this day, the Carborundum field engineer showed 7 positives on rectal swab (6 Polio, 1 Coxsackie B-4).

[g] Polio 1

[h] Polio 2

[i] Site H = effluent from open reservoir in Alexandria used by the Virginia American Water Company to supplement flow during periods of peak demand.

[j] See Tables 11 and 12.

APPENDIX D

Comparative Sampling Program:

Code Numbers Assigned to Concentrates Prepared by California State Department of Health Virus and Rickettsial Laboratory

TABLE D-1. CODE NUMBERS ASSIGNED TO VIALS SENT BY CALIFORNIA STATE HEALTH DEPARTMENT (CSHD), RECODE NUMBERS GIVEN UPON RECEIPT AT FAIRFAX COUNTY WATER AUTHORITY, AND SECOND RECODE NUMBERS ASSIGNED FOR CONCENTRATES SUPPLIED BY THE TWO VIRUS GROUPS INVOLVED IN THE COMPARATIVE STUDY (EPA AND THE CARBORUNDUM COMPANY)

CSHD Code No.	Virus Titer, Total PFU & Virus Type	Re-Code No.	Test[a] No.	Carborundum Concentrate, Number Assigned at FCWA[b]		EPA Concentrate Number Assigned at FCWA[b]	
				Carbo. Analyzed[c]	EPA Analyzed[c]	Carbo. Analyzed[c]	EPA Analyzed[c]
1-24	53	181	11	2063	1600	2213	1849
1-46	Polio 1	146	17	1166	2079	1560	2721
1-83		150	6	2498	1807	2872	1856
1-52		110	5	2206	1243	2999	1616
1-72		120	22	1606	2600	1721	2451
2-21		291	27	1069	2143	1071	2426
2-49		200	32	1706	2843	1599	2782
2-80		229	33	1065	2533	1515	2112
1-32	250	107	12	2210	1749	2691	1179
2-59	Polio 2	284	34	1705	2566	1219	2586
1-81	300	103	2	2428	1858	2809	1629
1-54	Polio 1	159	21	1549	2969	1684	2014
1-12		169	4	2905	1309	2564	1306
1-90		117	9	2738	1537	2440	1713
1-97		199	24	1927	2903	1012	2425
1-15		114	8	2119	1944	2418	1033
1-02		157	23	1411	2542	1006	2584
1-05		191	20	1688	2953	1130	2686
2-06		273	26	1377	2789	921	2579
2-22		265	30	1565	2531	1399	2810
2-50		236	31	1818	2537	1602	2740
2-75		219	35	1168	2784	1182	2494
1-56	2.9 X 10^6 Polio 2	148	1	2544	1556	2394	1334
2-94		222	28	1100	2366	862	2110
1-19	Blank	101	15	1703	2019	1997	2197
1-04		160	16	1581	2705	1867	2463
1-85		162	18	1612	2887	1191	2620
1-70		153	19	1782	2922	1604	2945
1-26		196	7	2310	1163	2167	1763
1-42		119	13	2585	1775	2412	1816
1-66		164	10	2134	1413	2884	1700
1-76		116	3	2956	1421	2272	1679
1-92		111	14	1379	2952	1375	2962
2-47		207	29	1751	2281	1277	2174
4-84		468	25	1635	2083	1461	2230

[a] Test Nos. 1-25: Vial contents diluted in thiosulfate reservoir; Nos. 26-35, in 260-gallon dilution tank filled with pasteurized water.

[b] No. assigned after concentrates returned to FCWA by the respective laboratories

[c] Carborundum labs at Univ. New Hampshire; EPA labs in Cincinnati

APPENDIX E

Results of Swab Tests

TABLE E-1. VIRUS DATA ON PERSONNEL

		Swabs		Virus, PFU	
Date	Lab No.	From	Description	BGM	PMK
1976					
6/21	703	McGee	Rectal	0	0
	704	McGee	Throat	0	0
	705	Saunders	Rectal	0	0
	706	Saunders	Throat	0	0
8/13	775A	George	Throat	0	0
	775B	George	Rectal	0	0
8/16	774A	Thacker	Throat	0	0
	774B	Thacker	Rectal	0	0
9/2	812B	McGee	Rectal	[a]7	0
	812A	McGee	Throat	0	0
9/8	813B	Saunders	Rectal	0	0
	813A	Saunders	Throat	0	0
9/22	817	McGee	Throat	0	0
	818	McGee	Rectal	0	0
9/30	893	McGee	Rectal	0	0
	892	McGee	Throat	0	0
	897	Saunders	Rectal	0	0
	896	Saunders	Throat	0	0
10/5	894	McGee	Throat	0	0
	895	McGee	Rectal	0	0
10/14	899	Thacker	Rectal	0	0
	898	Thacker	Throat	0	0
10/15	891	McGee	Rectal	0	0
	890	McGee	Throat	0	0
	889	Saunders	Throat	0	0
12/3	[b]1058-59	Saunders	Rectal	0	0
	[b]1060-61	Saunders	Throat	0	0
	1062	McGee	Rectal	0	0
	1063	McGee	Throat	0	0
	1064	Thacker	Rectal	0	0
	1065	Thacker	Throat	0	0
	1066	Hoehn	Rectal	0	0
	1067	Hoehn	Throat	0	0
	1068	Grizzard	Rectal	0	0
	1069	Grizzard	Throat	0	0
12/9	1070	Saunders	Rectal	0	0
	1071	Saunders	Throat	0	0
	1072	McGee	Rectal	0	0
	1073	McGee	Throat	0	0
	1074	Thacker	Rectal	0	0
	1075	Thacker	Throat	0	0

-Continued-

TABLE E-1 (continued)

		Swabs		Virus, PFU	
Date	Lab No.	From	Description	BGM	PMK
12/17	1076	McGee	Rectal	0	0
	1077	McGee	Throat	0	0
	1078	Saunders	Rectal	0	0
	1079	Saunders	Throat	0	0
	1080	Thacker	Rectal	0	0
	1081	Thacker	Throat	0	0
1977					
2/15	1243	Thacker	Rectal	0	0
	1244	Thacker	Throat	0	0
	1247	McGee	Rectal	0	0
	1248	McGee	Throat	0	0
	1173	Cameron	Rectal	0	0
	1172	Cameron	Throat	0	0
	1245	Saunders	Rectal	0	0
	1246	Saunders	Throat	0	0
3/1	1197	Thacker	Rectal	0	0
	1196	Thacker	Throat	0	0
	1194	Saunders	Rectal	0	0
	1195	Saunders	Throat	0	0
3/21	1241	Thacker	Rectal	0	0
	1242	Thacker	Throat	0	0
	1239	Saunders	Rectal	0	0
	1240	Saunders	Throat	0	0
6/6	1519	McConnaghy	Rectal	0	0
	1520	McConnaghy	Throat	0	0
6/7	1521	Thacker	Rectal	0	0
	1522	Thacker	Throat	0	0
	1523	Saunders	Rectal	0	0
	1524	Saunders	Throat	0	0

[a] 6 Polio 1 and 1 Coxsackie B-4

[b] Duplicate Samples

TABLE E-2. VIRAL ASSESSMENT OF RECTAL SWABS COLLECTED FROM EPA PERSONNEL ASSIGNED TO THIS PROJECT

Name	Specimen Collection Date	Cell Type PMK	Cell Type BGM
Akin	11/29/76	-[a]	-
Brashear	11/29/76	-	-
	1/17/77	-	-
Mayhew	11/29/76	-	-
	1/14/77	-	-
Stetler	11/29/76	-	-
	1/14/77	-	-
Waltrip	12/03/76	-	-
	1/17/77	-	-

[a] - = no viruses isolated by CPE method over 7-day observation period

*U.S. GOVERNMENT PRINTING OFFICE: 1981 341-082/230 1-3